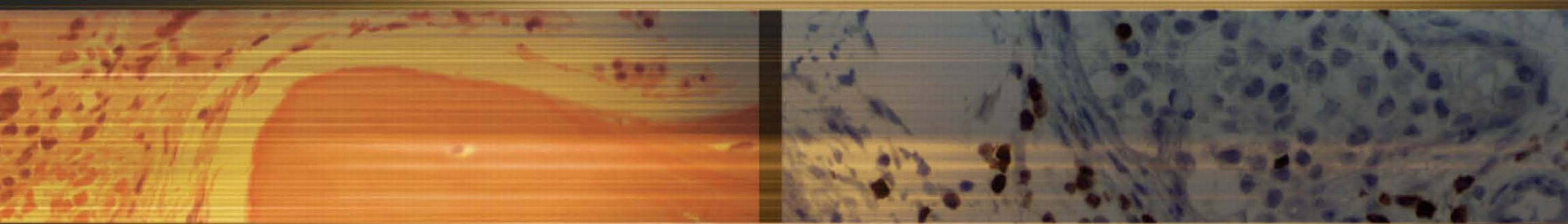
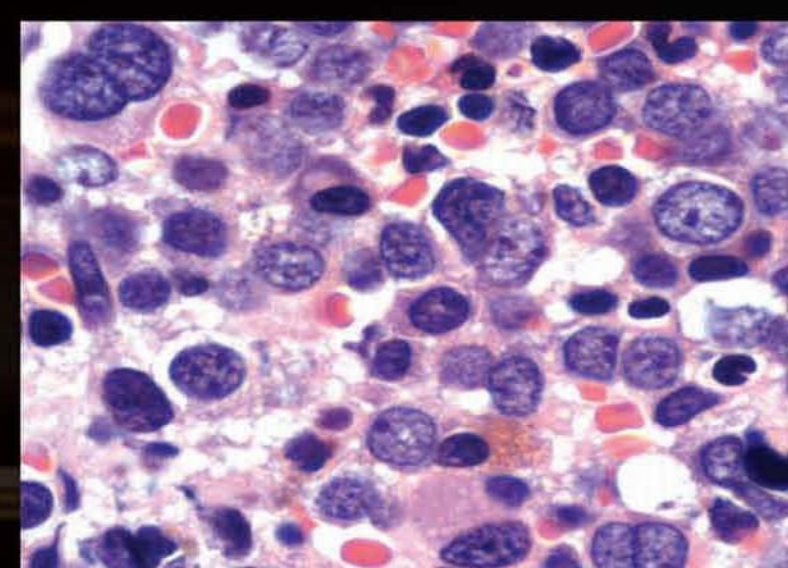
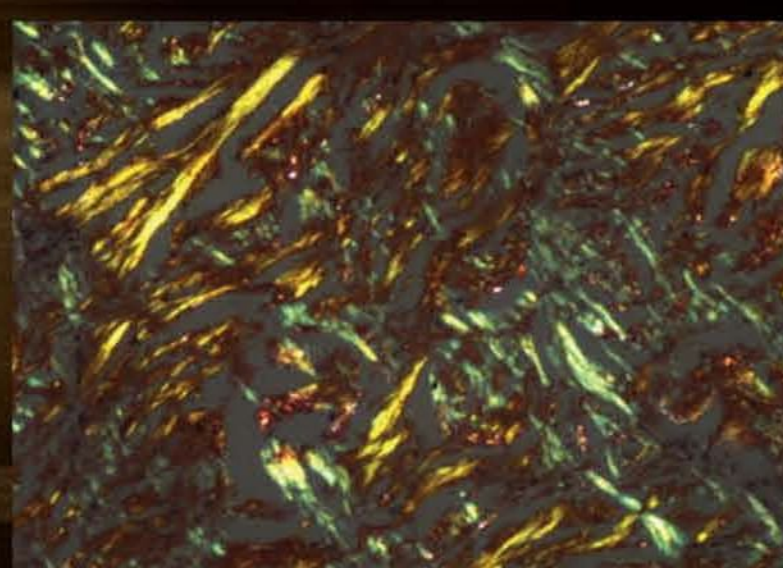
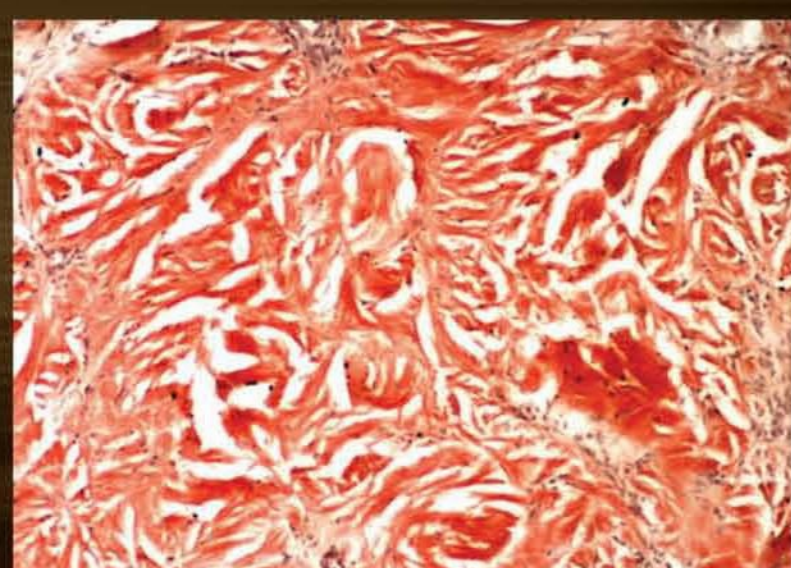


Flow Cytometry, Immunohistochemistry, and Molecular Genetics for Hematologic Neoplasms



S E C O N D E D I T I O N



TSIEH SUN

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Flow Cytometry, Immunohistochemistry, and Molecular Genetics for Hematologic Neoplasms

SECOND EDITION

Tsieh Sun, MD

Department of Hematopathology

UT MD Anderson Cancer Center

Houston, Texas



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10 9 8 7 6 5 4 3 2 1

This book is dedicated to my wife, Sue, for her constant support, patience and understanding.

Since the publication of the last edition of this book, tremendous progress has been made in the field of hematopathology. It involves the diagnosis, prediction of prognosis, and monitoring of treatment for hematopoietic neoplasms. While making an accurate diagnosis by pathologists has been the major goal in previous years, clinicians are no longer satisfied with a simple diagnosis nowadays. In this new era, detection of minimal residual diseases and risk stratification for the guidance of treatment have become mandatory issues. To meet these demands, a broad approach to the investigation of hematologic neoplasms is a norm not only for the large medical centers but also for smaller institutions. In other words, besides knowing how to make a morphologic diagnosis, the hematopathologist must also know how to utilize flow cytometry, immunohistochemistry, and molecular genetics to provide answers to clinical questions. Among all these parameters, molecular genetics has emerged as the most reliable diagnostic tool as well as the major criterion for the stratification of risk groups. This view is reflected in the 2008 World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues. In this new scheme, molecular genetics is greatly emphasized in each tumor entity, and many karyotype-defined new entities have been delineated. It appears that the gene products not only help in the subclassification of lymphomas and leukemias and the prediction of their prognosis but also provide potential targets for inhibitory treatment.

The knowledge of molecular genetic principles is no longer a theoretical curiosity but a necessity for the understanding of tumorigenesis. For instance, deregulation of signal transduction pathways is the major mechanism of pathogenesis in various lymphomas and leukemias. The interaction of oncogenes, transcription factors, and microRNAs is another intriguing phenomenon to be reckoned with. In recent years, we have also learned that molecular genetic study of tumor cells alone is biased, as the prognosis of a patient frequently depends upon the microenvironment and the epigenetics of the tumor. Fortunately, a plethora of new techniques, particularly the microarray-based tests, provide a great help for the identification and understanding of all these factors. Accordingly, in this new edition, not only are the number of cases (11 additions) and illustrations (200 additions) expanded but the content also covers the new knowledge in molecular genetics with the discussion of the basic principles in the introductory chapters and the addition of new and potentially useful molecular genetic studies in individual cases. While comprehensive coverage may not be possible, the reader will find that this book facilitates a better understanding of the current literature in a clear and concise manner.

Tsieh Sun, MD

Preface to the First Edition



In recent years, hematopathology has become increasingly complicated. The complexity of hematopathology is due mainly to two reasons. First, the new classifications of lymphomas and leukemias (namely, the Revised European-American Classification of Lymphoid Neoplasms and the World Health Organization Classification) furnish a great variety of disease-specific entities not only on the basis of morphology, on which the older classifications were based, but also in consideration of the immunophenotype, molecular genetic findings, and clinical manifestations. Second, there is a plethora of new techniques emerging in recent years, which not only helps in the accuracy of diagnosis but also provides guidelines for the treatment and indicators for prognosis. In the cytogenetic area, fluorescence in situ hybridization has greatly expanded the versatility of traditional karyotyping. In the field of molecular biology, polymerase chain reaction (PCR) has gradually replaced the time-consuming Southern blot technique. The branching of PCR into quantitative PCR and reverse transcriptase PCR (RT-PCR) brings molecular biology to a new horizon. In situ hybridization and microarrays have gradually entered into the immunohistochemical laboratories of large medical centers. Finally, the recent development of gene expression profiling provides a power tool for stratification of lymphomas and leukemias and is highly promising for the diagnosis, prediction of prognosis, and guidance of treatment for hematologic neoplasms.

Despite of all the exciting technological advances, immunophenotyping remains the mainstay in the routine diagnosis of hematologic neoplasms. Most laboratories use immunohistochemistry for immunophenotyping, because it is more convenient for morphologic correlation and it can be easily handled by a histology laboratory. However, flow cytometry provides timely, quantitative information and frequently a definitive distinction between malignant and benign lesions (e.g., information of light-chain restriction). Whereas flow cytometry is more helpful in diagnosing leukemias and generates prognostic predictors in some entities (e.g., CD38 and ZAP 70 for chronic lymphocytic leukemia), immunohistochemistry is more useful in diagnosing lymphomas with small numbers of tumor cells (e.g., Hodgkin lymphoma, T-cell-rich B-cell lymphoma) and provides some specific markers that are not available for flow cytometry (e.g., cyclin D1 for mantle cell lymphoma and bcl-6 for lymphomas of follicular center cell origin). Although the utilization of both techniques will undoubtedly yield a complete picture and more definitive diagnoses

of hematologic tumors, concerns of cost restraint and specimen size frequently limit the luxury of choice. The major purpose of this book is to discuss the pros and cons of these two techniques in individual diseases so that the reader can make an intelligent choice to achieve an accurate diagnosis.

Whereas immunophenotyping may help diagnosing most cases of hematologic neoplasms, molecular cytogenetic techniques are mandatory for the diagnosis of a few tumors, such as Burkitt lymphoma and chronic myelogenous leukemia. In some tumors, the genotype is decisive in the choice of therapy. For instance, if the karyotype in a case of acute promyelocytic leukemia is not t(15;17) but its variant, the patient may not respond to the therapy of all-trans-retinoic acid, and arsenic trioxide may have to be used. Cytogenetic studies are also important in cases with acute myeloid leukemia, myelodysplastic syndromes, and chronic myeloproliferative disorders, because the karyotypes in many cases dictate the clinical manifestations and the prognosis. The prognostic prediction, in turn, affects the therapeutic decision. In addition, for the detection of minimal residual disease after therapy, molecular genetic techniques are frequently more sensitive than immunophenotyping. Unfortunately, those sophisticated molecular genetic techniques are only available in limited numbers of reference laboratories, and they are frequently costly. Therefore, guiding the reader to select these new techniques in supplementing immunophenotyping is the second important task of this book.

Most hematopathology textbooks emphasize the application of immunohistochemistry, whereas flow cytometry textbooks seldom mention this technique. This book will provide a balanced view on both areas and a convenient way for the reader to study both subjects side by side. As case review is the most efficient way of learning, this book includes a large number of cases, covering all important clinical entities, with detailed clinical history and flow cytometric and immunohistochemical findings preceding the discussion of the individual disease. Abundant color illustrations of immunohistochemical stains, flow cytometric histograms, and tables summarizing diagnostic features and comparing similar diseases are furnished to facilitate the reader's understanding. It is the wish of the author that this book will become a handy guidebook for the hematopathologists and clinical hematologists/oncologists in their busy daily practice.

Tsieh Sun, MD



Acknowledgments



The second edition of Flow Cytometry, Immunohistochemistry, and Molecular Genetics for Hematologic Neoplasms was prepared at the Department of Hematopathology, University of Texas MD Anderson Medical Center, Houston,

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Preface v

Preface to the First Edition vii

Acknowledgments ix

CHAPTER 1	General Introduction	1
CHAPTER 2	Principles of Flow Cytometry	4
CHAPTER 3	Principles of Immunohistochemistry	16
CHAPTER 4	Molecular Genetics	25
CHAPTER 5	Classification of Hematologic Neoplasms	38
CHAPTER 6	Clinical Application	51
CASE 1	Chronic Myelogenous Leukemia	60
CASE 2	BCR-ABL1–Negative Myeloproliferative Neoplasms	69
CASE 3	Myelodysplastic Syndromes	77
CASE 4	Myelodysplastic/Myeloproliferative Neoplasms	87
CASE 5	Acute Myeloid Leukemia with t(8;21)(q22;q22)	95
CASE 6	Acute Myeloid Leukemia with inv(16)(p13q22) or t(16;16)(p13;q22)	101
CASE 7	Acute Promyelocytic Leukemia with t(15;17)	107
CASE 8	Acute Myeloblastic Leukemia without Maturation (General Introduction of Acute Myeloid Leukemia)	115
CASE 9	Acute Myeloblastic Leukemia with Maturation	125
CASE 10	Acute Myelomonocytic Leukemia	130
CASE 11	Acute Monoblastic and Monocytic Leukemia (M5) and 11q23 (Mixed Lineage Leukemia) Abnormalities	135
CASE 12	Acute Erythroid Leukemia	141
CASE 13	Acute Megakaryoblastic Leukemia	148
CASE 14	Myeloid Sarcoma	156
CASE 15	Blastic Plasmacytoid Dendritic Cell Neoplasm	163
CASE 16	Mixed Phenotype Acute Leukemia	168



CASE 17	B-Lymphoblastic Leukemia/Lymphoma	173
CASE 18	T-Lymphoblastic Leukemia/Lymphoma	181
CASE 19	Chronic Lymphocytic Leukemia of B-Cell Lineage	191
CASE 20	Chronic Lymphocytic Leukemia of T-Cell Lineage	199
CASE 21	Small Lymphocytic Lymphoma	205
CASE 22	Prolymphocytic Leukemia	212
CASE 23	Lymphoplasmacytic Lymphoma	218
CASE 24	Plasma Cell Myeloma and Plasmacytoma	226
CASE 25	Hairy Cell Leukemia	238
CASE 26	Splenic B-Cell Marginal Zone Lymphoma	247
CASE 27	Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue	255
CASE 28	Nodal Marginal Zone Lymphoma	264
CASE 29	Follicular Lymphoma	270
CASE 30	Primary Cutaneous Follicle Center Lymphoma	280
CASE 31	Mantle Cell Lymphoma	285
CASE 32	Diffuse Large B-Cell Lymphoma	294
CASE 33	T-Cell/Histiocyte-Rich Large B-Cell Lymphoma	302
CASE 34	Primary Mediastinal (Thymic) Large B-Cell Lymphoma	307
CASE 35	Intravascular Large B-Cell Lymphoma	314
CASE 36	Burkitt Lymphoma/Leukemia	318
CASE 37	T-Cell Large Granular Lymphocytic Leukemia	329
CASE 38	Natural Killer Cell Lymphoma/Leukemia	336
CASE 39	Adult T-Cell Leukemia/Lymphoma	345
CASE 40	Hepatosplenic T-Cell Lymphoma	352
CASE 41	Mycosis Fungoides and Sézary Syndrome	358
CASE 42	Subcutaneous Panniculitis-Like T-Cell Lymphoma	366
CASE 43	Peripheral T-Cell Lymphoma, Not Otherwise Specified	371
CASE 44	Angioimmunoblastic T-Cell Lymphoma	378



CASE 45	Anaplastic Large-Cell Lymphoma	383
CASE 46	Primary Cutaneous Anaplastic Large-Cell Lymphoma	393
CASE 47	Hodgkin Lymphoma	398
CASE 48	Post-transplant Lymphoproliferative Disorders	409
CASE 49	Langerhans Cell Neoplasm	415
CASE 50	Thymoma	420

Index 427

General Introduction

1

With the progress in subclassification of hematologic neoplasms and the refinement in treatment of these tumors, diagnosis of lymphoma and leukemia has become increasingly complicated. The World Health Organization (WHO) classification of tumors of hematopoietic and lymphoid tissues not only relies on morphology of the tumors but also requires immunophenotyping and molecular genetic studies for an accurate diagnosis. Immunophenotyping can be achieved either by flow cytometry (FC) or immunohistochemistry (IH) techniques. Although each of these two techniques has its advantages and disadvantages, each is complementary to the other, and they should be used together. In recent years, molecular genetic studies have played an increasingly important role, not only for the diagnosis but also for prediction of the prognosis. This book will discuss the pros and cons in the application of these techniques as well as their respective roles in diagnosing various hematologic neoplasms.

The flow cytometer has been hailed as a new product of technical revolution, but the concept of FC has existed for >50 years, and cell counters have been used extensively for >20 years. Nevertheless, the emergence of the fluorescence detector in the new generation of cytometers greatly enhanced their versatility. The availability of a great variety of monoclonal antibodies finally pushed FC to the forefront. The outbreak of acquired immunodeficiency syndrome (AIDS) incidentally accelerated the acceptance of flow cytometers as routine laboratory instruments because of the tremendous demands for testing the helper:suppressor T-cell ratio as a screening technique in the early epidemic of AIDS. However, flow cytometer has been promptly adopted by hematology/oncology laboratories as a routine tool because most monoclonal antibodies are cell lineage- or developmental stage-specific for blood cells.

FC has the advantage of being more efficient, sensitive, accurate, and reproducible than manual techniques. With FC, multiple specimens can be simultaneously processed with a panel of 10 or more monoclonal antibodies, and tests can be completed within several hours. When there is a sufficient specimen, FC counts 3,000 to 5,000 cells for the study of each antigen, compared with 100 to 200 cells counted in manual techniques. The examination of large

numbers of cells enhances the sensitivity and accuracy of FC and makes it possible to detect small numbers of neoplastic cells. The percentage of various cell groups obtained by FC is highly reproducible and is thus comparable between different laboratories. However, the major merit of FC is its capability of simultaneously measuring multiple parameters (forward light scatter, side scatter, and fluorescence), and the data thus obtained can be stored for further analysis. In addition, the electronic gating gadget enables the study of separate cell groups without requiring tedious isolation techniques.

The major limitations of FC, at this stage, are the high cost of the instrument, the special skill required to operate it, and the lack of a morphologic correlation with the markers. The failure of the machine to distinguish a normal cell from a tumor cell leads to the indiscriminate counting of both populations; thus a wrong conclusion may be drawn if not enough tumor cells or parameters are analyzed. However, with the availability of increasing numbers of specific monoclonal antibodies and fluorochromes, and the emergence of four- to eight-color analyzers, the function of FC in terms of diagnosis and therapeutic monitoring has been markedly improved in recent years.

The current simplification of this automated instrument makes it feasible to use as a routine laboratory procedure, not only in large medical centers but also in medium-sized hospitals. Although it is now technically possible for clinical laboratories to use flow cytometers, the lack of expertise in interpreting the results still hampers the broad use of this instrument. It is apparent that a well-illustrated guidebook with detailed clinical cases is needed to help flow cytometer workers to understand how to apply FC to identify malignant cells and to diagnose hematologic neoplasms.

However, the more popular technique for immunophenotyping is IH for obvious reasons. IH can be conveniently performed in a histology laboratory with a minimal investment in equipment and reagents, while very expensive equipment for IH is also available. It can be done on frozen as well as permanent sections. The feasibility of performing IH on archived material retrospectively is a great advantage of IH. Nevertheless, the major merit of IH is

TABLE 1.1

Comparison of Immunohistochemistry and Flow Cytometry

	Immunohistochemistry	Flow Cytometry
Morphologic correlation	Excellent	No visual correlation
Specimen required	Paraffin and frozen sections	Blood, marrow, body fluid, fine needle aspirates, and fresh solid tissues
Available antibodies	Limited but gradually expanded	Abundant
Dual stain on same cells	Limited	5- to 8-color staining
Quantitation	Estimation with imaging techniques	Accurate and reproducible
Distinction between surface and cytoplasmic staining	Difficult	Easy
Turnaround time	4 h after hematoxylin and eosin–stained section is examined	3 h

the direct correlation of morphology with markers, which makes surgical pathologists more confident in interpreting the results leading to a diagnosis.

At this stage, the application of IH is limited by the availability of monoclonal antibodies that can be used for histologic staining. After fixation and embedding, many antigenic epitopes are altered so that they can no longer react to most antibodies that are used for FC. However, in recent years, monoclonal antibodies detecting the hidden antigenic radicles have increased rapidly and many antibodies for gene product proteins (e.g., cyclin D1, BCL-2, BCL-6) are particularly useful.

One of the major drawbacks of IH is its inability to demonstrate surface immunoglobulins on most B cells and thus the inability to demonstrate a clonal B-cell population in most cases of lymphomas. The antigen-retrieving technique using a microwave oven may help to detect immunoglobulins in some tumors, especially in those with abundant cytoplasm, such as immunoblastic lymphoma and plasma cell neoplasms. The inability to distinguish surface from cytoplasmic antigens is another drawback. For instance, cytoplasmic CD3 is present in early T-cell stage (thymocytes), and surface CD3 is detected in mature T-cell stage (peripheral T cells). When a tumor is stained positive for CD3 by IH, the developmental stage of the tumor cells cannot be pinpointed. The same is true for the distinction between natural killer (NK) cells and NK-like T cells.

There are other limitations of IH. Multiple staining cannot be performed on the same cells. Therefore, the characterization of tumor cells by IH is not as comprehensive as by FC, which can detect two to four antigens on the same cells. IH using sequential horseradish peroxidase and alkaline phosphatase can label two different antibodies, but it is difficult to demonstrate two antigens on the same cell.

Another limitation of IH is the inability to quantify the percentage of tumor cells for therapeutic monitoring. The imaging technique may achieve the effect of semiquantitation, but it is tedious and the information is not as accurate

as that obtained from FC. Finally, IH is usually ordered after examination of the hematoxylin and eosin–stained sections, so that a conclusion cannot be made until the third day after the receipt of the specimen. The turnaround time of FC is approximately 3 hours, and the results can be obtained on the day the specimen is received. The advantages and disadvantages of IH and FC are listed in Table 1.1.

In the molecular genetic front, many innovative techniques have emerged in recent years. The fluorescence in situ hybridization technique not only is supplementary to but also supersedes conventional karyotyping in detecting cytogenetic abnormalities. The advances in molecular biology techniques are amazingly speedy. The polymerase chain reaction almost totally replaces Southern blotting technique for genotyping. On the basis of the microarray principle, gene expression profiling, comparative genomic arrays, and single nucleotide polymorphism arrays are created and many cryptic genetic aberrations can now be detected. In addition to genetic factors, many epigenetic changes also play an important role in tumorigenesis and become ideal targets for treatment.

The importance of correlating morphologic findings with clinical features, immunophenotype, and molecular genetics can never be overemphasized. Therefore, the core of this book is the presentation of 50 clinical cases with pertinent information to provide the reader with a comprehensive concept of hematologic neoplasms and the role of immunophenotype and molecular genetics in diagnosis, classification, and treatment of these disorders. Case review is a most efficient way of learning and is probably a modern trend in learning methods. In each section, a case history and immunophenotypic findings are presented, followed by a concise discussion in morphology, immunophenotyping, molecular genetics, and clinical presentation.

To make this book understandable by people from different disciplines (clinicians, anatomic pathologists, clinical pathologists, and other clinical laboratory scientists) who share an interest in immunophenotyping and molecular genetics, three introductory chapters are

included to familiarize the reader with the basic principles of FC, IH, and molecular genetics. Preceding the case presentations is a chapter on classification of hematologic neoplasms. There have been many classifications of this group of tumors, but the WHO classification is now universally accepted. The principle of classification is again based on clinical presentation, morphology, immunophenotype, and molecular genetics. For instance, on the basis of clinical presentation, tumors can be divided into predominantly disseminated lymphoma and/or leukemia, primary extranodal lymphomas, and predominantly nodal lymphoma. Immunophenotyping can divide hematologic neoplasms into different maturation stages, such as progenitor B cell, pre-B cell, immature B cell, mature B cell, activated B cell, and plasma cell. Based on the origin of intranodal B-cell differentiation, tumors can be assigned to germinal center, mantle zone, marginal zone, and parafollicular perisinusoidal areas. Finally, according to the presence or absence of mutation of the variable region of immunoglobulin heavy-chain gene, lymphomas can be divided into pregerminal center, germinal center, and postgerminal center groups.

Currently, there are many monoclonal antibodies available for identification of different cell lineages or developmental stages. The selection of appropriate antibodies is the important first step for immunophenotyping, not only because of cost containment but also because of the limited size of specimens. As using a general panel to cover multiple cell lineage or all clinical situations is impossible to set up, one may select to use a two-tiered approach or targeted approach for monoclonal antibody selection. A two-tiered approach uses a screening panel to determine the final panel of monoclonal antibodies. The targeted approach is based on either the clinical diagnosis or morphology (by examining the tissue imprints, blood or bone marrow aspirate smears) to select the monoclonal antibody panel. To guide the reader in a targeted approach, abundant morphologic illustrations from specimens of blood, bone marrow, lymph node, spleen, and other soft tissues are provided in this book.

The goal of this book is to serve as a handy laboratory guide for immunophenotyping and molecular genetic studies of hematologic neoplasms. The users may want to

compare our cases with their own to find out what they need to study and to avoid omission of some important tests. For this purpose, the summary table at the end of each case study should be used as a quick reference. For those who are not familiar with certain theories or techniques, the introductory chapters may serve as short refresher courses. For many special aspects that are not covered by this book, the reader is referred to Suggested Readings.

SUGGESTED READINGS

1. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 7th ed. Philadelphia, PA: Elsevier; 2011.
2. Carey JL III, McCoy P Jr, Keren DF. Flow Cytometry in Clinical Diagnosis. Chicago, IL: ASCP Press; 2010.
3. Dabbs DJ, ed. Diagnostic Immunohistochemistry. Theranostic and Genomic Applications. 3rd ed. Philadelphia, PA: Saunders; 2010.
4. Elias JM. Immunohistopathology: A Practical Approach to Diagnosis. 2nd ed. Chicago, IL: ASCP Press; 2003.
5. Foucar K, Reichard K, Czuchlewski D. Bone Marrow Pathology. 3rd ed. Chicago, IL: ASCP Press; 2010.
6. Ioachim HL, Medeiros LJ. Ioachim's Lymph Node Pathology. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2009.
7. Jaffe ES, Harris NL, Vardiman JW, et al. Hematopathology. Philadelphia, PA: Saunders; 2011.
8. Knowles DM, ed. Neoplastic Hematopathology. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001.
9. Nguyen D, Diamond LW, Braylan RC. Flow Cytometry in Hematopathology: A Visual Approach to Data Analysis and Interpretation. 2nd ed. Totowa, NJ: Humana Press; 2007.
10. Shapiro HM. Practical Flow Cytometry. 4th ed. Hoboken, NJ: John Wiley & Sons; 2003.
11. Sun T. Atlas of Hematologic Neoplasms. Dordrecht, The Netherlands: Springer; 2009.
12. Sun T. Comparison of immunohistochemistry and flow cytometry in immunophenotyping of hematologic neoplasms. J Histotechnol. 2004;27:101–109.
13. Sun T. Immunophenotyping of hematologic neoplasms by combined flow cytometry and immunohistochemistry. J Clin Ligand Assay. 2004;27:180–189.
14. Swerdlow SH, Campo E, Harris NL, et al. eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008.

2

Principles of Flow Cytometry

INSTRUMENTATION

A flow cytometer is versatile in its capability of measuring multiple parameters simultaneously. These parameters include the physical properties of cells (e.g., cell size and cytoplasmic granularity), surface membrane, cytoplasmic and nuclear antigens, and DNA–RNA contents of individual cells in a cell suspension. Surface, cytoplasmic, and nuclear antigens are detected by means of fluorochrome-conjugated antibodies. These antigens are thus the extrinsic properties of the cell. The physical properties of the cell are the intrinsic properties, because no exogenous reagents are added for their detection. These parameters are measured through an optical system, and the light signal thus generated is registered in an electronic system. The computer system is responsible for data storage, gating, and graphic display on a screen.

Although the Coulter counter can be considered a **flow** cytometer, the term **flow** cytometer is usually reserved for cell counters with **fluorescence** detectors, such as the FACSCanto II (Becton Dickinson) (Fig. 2.1) and the Cytomics FC500 (Beckman Coulter, Inc.) (Fig. 2.2). The design of the optical, electronic, and computer systems may be somewhat different in various **flow** cytometers, but they are constructed with essentially the same principles.

With the change of light source from mercury arc to laser, the cooling system from water cooled to air cooled, and the computer system from simple to sophisticated, the size of the **flow** cytometer has become smaller (tabletop) and its function has expanded in terms of data storage, number of **fluorochromes** used, and graphic and data display. However, the basic components of a **flow** cytometer remain the same: the **fluid** transport system, the optical system, the electronic system, and the computer system (1–8).

FLUID TRANSPORT SYSTEM

The fluid transport system starts with a sample receiving area where test tubes containing patients' specimens and controls are placed in a carousel. The cell suspension is aspirated by means of differential air pressure or

vacuum into a tubing system leading to the flow chamber (Fig. 2.3). When in the flow chamber, which is a conical nozzle, the specimen is surrounded by a cell-free stream of sheath fluid, producing a laminar flow configuration. The outer sheath fluid forces the cells in the sample to line up a single file. When the sample exits from the flow chamber through a narrow orifice, the flow velocity becomes markedly increased (about 1 to 10 m/s). The cell stream then meets the light source (mercury arc lamp or laser) at the light interception point in the sensing area, and electric or optical signals are generated.

OPTICAL SYSTEM

Electric signals are generated due to changes in electric resistance of the fluid when cells suspended in an electrically conducting medium pass through a light beam in the sensing area. Based on the fact that blood cells have lower electric conductivity than saline solution has, Wallace Coulter designed a cell counter that has dominated the American market for >30 years. The principle he used to measure cell volume is now called the Coulter Principle. This principle was also used in some older models of flow cytometers, such as the fluorescence-activated cell-sorting (FACS) analyzer (Becton Dickinson), which contains a mercury–cadmium arc lamp. The advantages of using mercury and xenon arc lamps are their low cost, ease of operation, and broad range of spectral output. The disadvantages are nonuniform radiance, low brightness, and instability of the arc. Because the disadvantages outweigh the advantages, mercury arc lamps are no longer used in the new models of flow cytometers.

Currently, all **flow** cytometers use lasers as the light source, and optical instead of electric signals are generated. Lasers can deliver intense, coherent (waves of light that are parallel and unidirectional), monochromatic light (single color or wavelength), thus resulting in low divergence and high brightness of the signals generated. The use of small air-cooled ion lasers has facilitated the development of smaller, less expensive, easier-to-install **flow** cytometers, such as the FACSCanto II and Cytomics FC500.

System with Fluidics Cart



BD

FIGURE 2.1 Photograph of FACSCanto II flow cytometer manufactured by Becton Dickinson. (From Becton Dickinson with permission.)

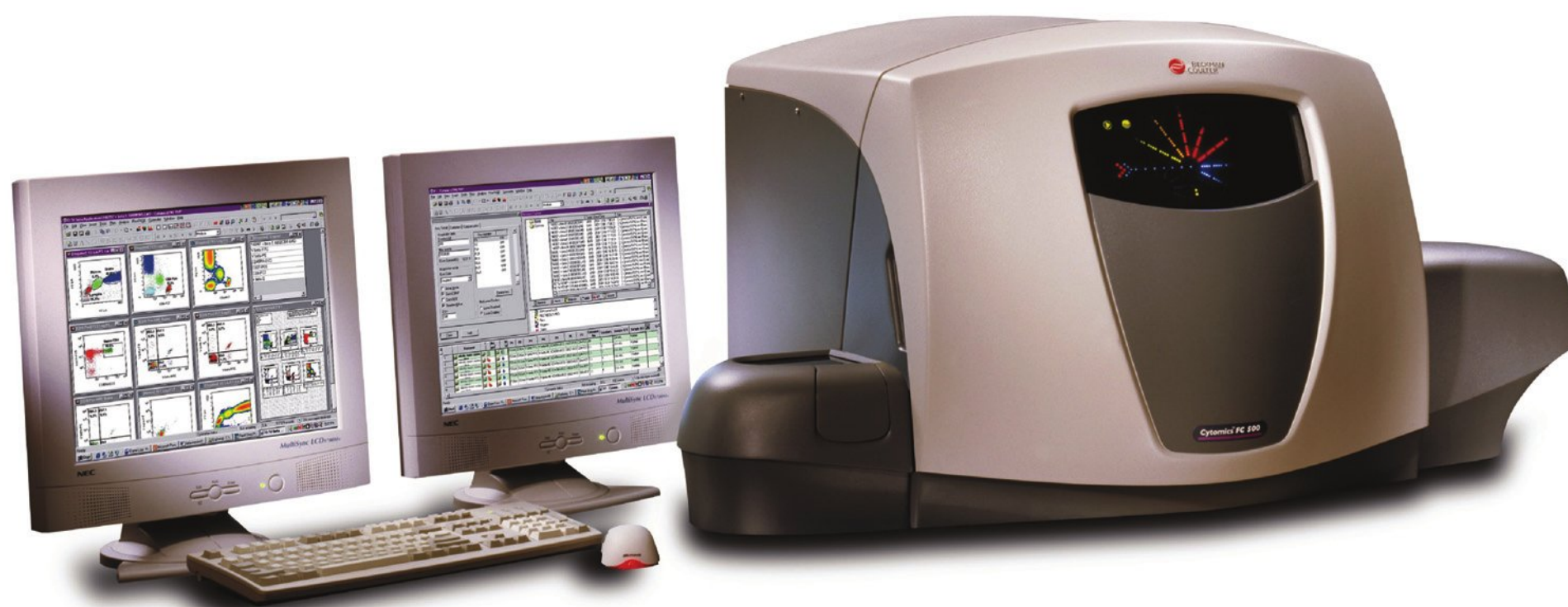


FIGURE 2.2 Photograph of Cytomics FC500 flow cytometer manufactured by Beckman Coulter. (From Beckman Coulter, Inc., with permission.)

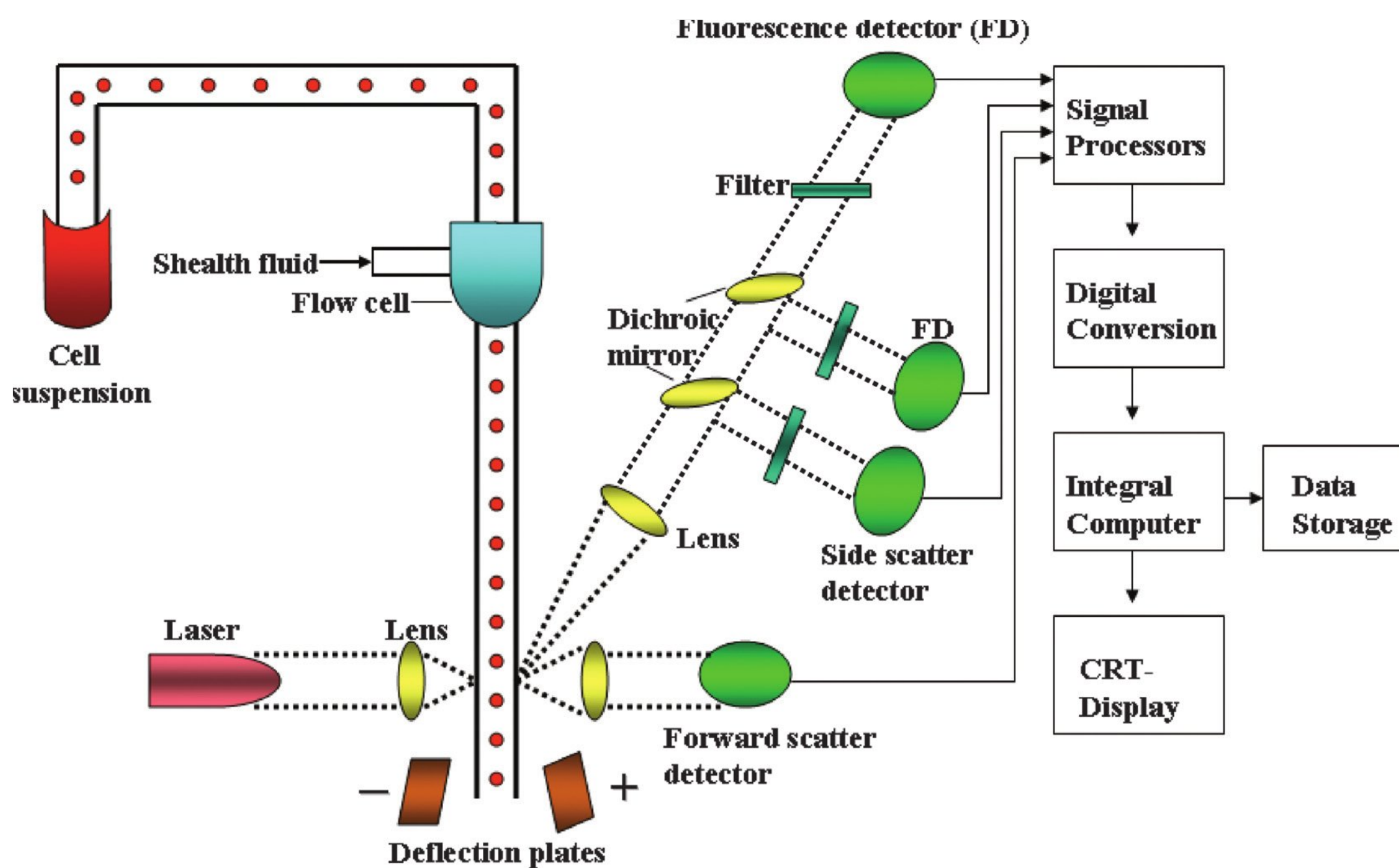


FIGURE 2.3 Basic structure of a flow cytometer showing the fluid transportation system, the optical system, the electronic system, and the cell sorter.

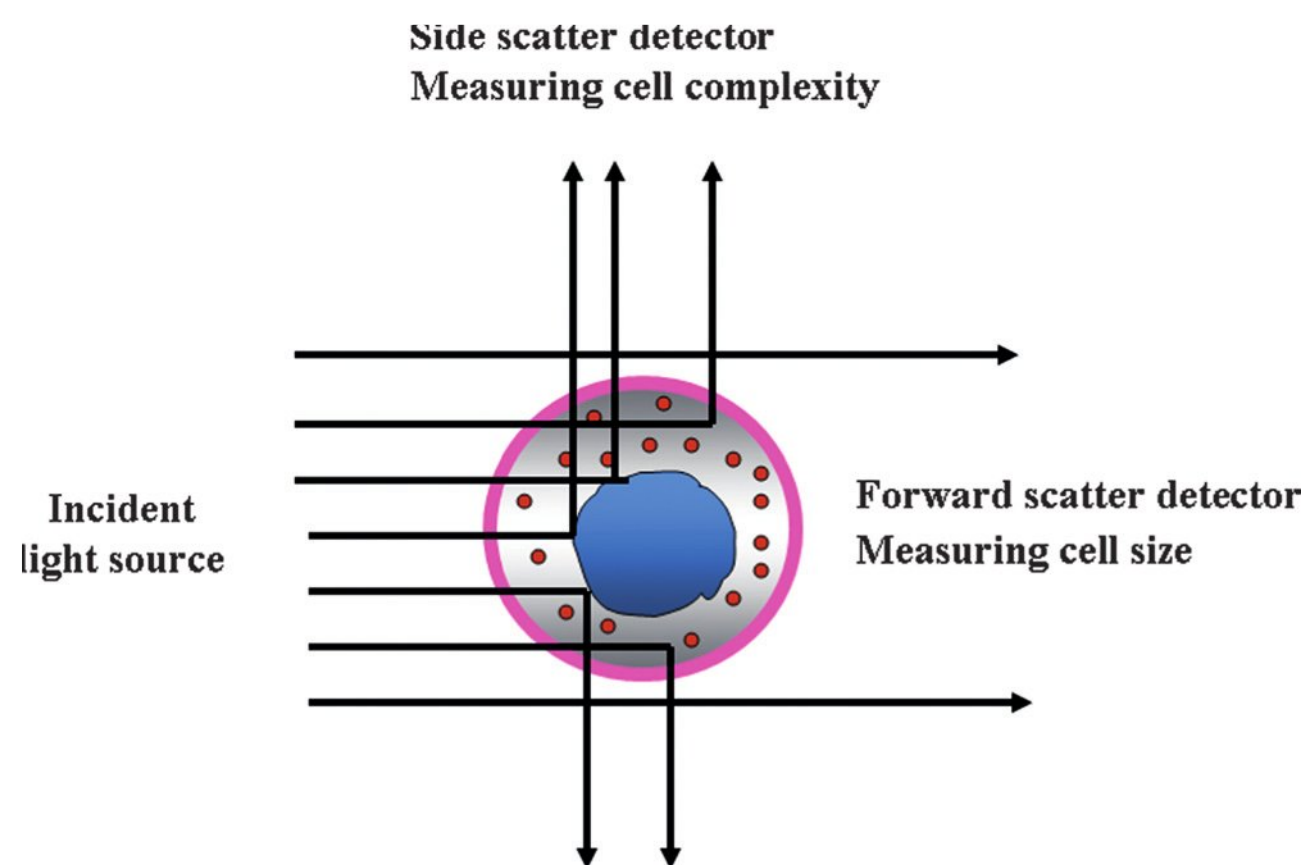


FIGURE 2.4 Schematic illustration showing the relationship of light scatter and cell size/structure.

The most frequently used optical signals are forward-angle (2 to 10 degrees) and right-angle (90 degrees) light scatter (Fig. 2.4). The former is proportional to cell size, and the latter is related to cell characteristics, such as cytoplasmic granularity and nuclear configuration.

Fluorescent Signals

The light scatter signals are usually used for screening. However, the characterization of cells is mainly based on their surface, cytoplasmic, and nuclear antigens, which are detected through a special optical signal: the fluorescent signal. Fluorescent signals are generated through fluorochrome-labeled antibodies that react specifically with various cell antigens, thus facilitating the identification of cell lineage, developmental stage, and special groups of tumor cells. Cellular DNA and RNA can be directly stained by intercalating agents, such as propidium iodide, which can bind DNA and RNA with the resultant generation of fluorescent signals.

Fluorochrome-labeled cells absorb incident light from the laser, resulting in emission of a longer wavelength, which is called fluorescence. The color of light emitted is a function of its wavelength (Table 2.1). As long as the

wavelengths emitted from the various fluorochromes do not overlap, the same specimen can be treated with 2 to as many as 10 fluorochrome-conjugated antibodies. The selection of fluorochrome is determined by the compatibility between the wavelength spectrum of the light source and the excitation ranges of the fluorochromes (Table 2.2). The most frequently used fluorochrome pair is fluorescein isothiocyanate (FITC) and phycoerythrin (PE).

Lens, Filter, and Mirror

A series of optical devices is used to direct the incident light, reflected light, and fluorescence to detectors. A beam-shaping lens is used to focus the laser down to a narrow beam waist of 20 to 100 nm in the sensing area. In multicolor analysis, separation of multicolored light is accomplished by the combined use of filters and dichroic mirrors. A short-pass filter allows only light of short wavelengths to pass and blocks that of the longer wavelengths. A long-pass filter acts the opposite way. Dichroic mirrors, in contrast, allow light of certain wavelengths to pass and reflect the light that is not allowed to pass.

ELECTRONIC SYSTEM

After traveling through all the filters and lenses, the photons of light impinge on the detectors and are converted into electrons (photoelectric effect). The detectors include photomultiplier tubes, which are commonly used for side-scatter and fluorescence signals, and photodiodes, which are used for absorption, extinction, and forward-scatter signals. The electronic signals or voltage pulses are analog signals in various magnitudes from 1 to 10 V. Analog signals may be processed as peak amplitude (height), integral (area), width (duration), or shape of the pulse and are expressed in either logarithmic or linear scales.

The analog signals are digitized in the analog-to-digital converters. The digital signal is a function of instrument resolution. A low-resolution instrument may have 256-channel resolution, whereas a high-resolution instrument may

TABLE 2.1
Wavelength and Color of Fluorochromes

Fluorochrome	Excitation max. (nm)	Emission max. (nm)	Color
Propidium iodide	488	620	Red
Peridinin chlorophyll	488	670	Red
Fluorescein isothiocyanate	494	517	Green
Phycoerythrin	495	576	Orange
Rhodamine	545	575	Orange
Texas Red	596	615	Red
Phycocyanin	620	655	Red
Allophycocyanin	620	660	Red
Cyanin 5	633	670	Red

TABLE 2.2 Wavelength of the Light Source and Choice of Fluorochrome Combination		
Light source	Excitation wavelength (nm)	Fluorochrome combination
Mercury arc lamp	485, 546	FITC, PE, RD1
Argon ion laser	488	FITC, PE, RD1
Dye laser	600	Texas Red, APC
Krypton ion laser	568, 647	APC
Helium–neon laser	633	APC

APC, allophycocyanin; FITC, fluorescein isothiocyanate; PE, phycoerythrin; RD1, rhodamine.

range over 1,024 channels. The last component of the electronic system is the pulse height analyzer, which analyzes the digital signals and quantifies them for computer display on an oscilloscope screen.

COMPUTER SYSTEM

The flow cytometer can be interfaced with an external computer system that performs three important functions.

List-mode storage: All parameters measured can be stored permanently on a floppy disk or temporarily on a hard disk. All data can be combined and analyzed later and can be printed in graphic form as a permanent record.

Gating: A gate is an electronic window that can be set with a cursor on the screen in a rectilinear or amorphous form to circumscribe a group of cells with similar characteristics (e.g., size and cytoplasmic granularity). Gating is performed to electronically isolate this special group of cells for analysis, avoiding the difficult task of purifying the cell population by biologic means. Depending on the software capability, several gates can be set so that information can be gathered on several populations of cells.

Graphic display: The computer can provide graphic displays in several forms, as described below.

Scattergram (Dot Plot, Cytogram)

This is a graphic display of dots as determined by two related parameters along the x- and y-axis. For instance, one can plot forward-angle light scatter against right-angle light scatter; each dot represents a single cell or event. On the basis of cell size (forward scatter) and cytoplasmic granularity (side scatter), the scattergram usually shows three distinct groups of cells in peripheral blood samples. The lymphocytes are the smallest (with no cytoplasmic granules); the monocytes are the largest; the granulocytes have the most cytoplasmic granules (Fig. 2.5). In lymph node specimens, the distinction between a group of large cells and a group of small cells frequently helps to separate lymphoma cells from reactive lymphocytes or a small-cell lymphoma from a mixed small- and large-cell lymphoma.

Single Histogram

This graph is usually used to display the number of cells (y-axis) versus fluorescence intensity (x-axis). When cells are stained with a fluorochrome-labeled antibody, a single histogram provides the percentages of positive and negative populations (Fig. 2.6). An isotopic-negative control should be used to determine the cutoffpoint between these two populations. For instance, if the monoclonal antibody is mouse immunoglobulin G (IgG), the mouse IgG with no specific antibody function should be used. When DNA stain is used, the single histogram can demonstrate the percentage of cells in different stages of the cell cycle and determine the ploidy of chromosomes with a normal control.

Contourgram (Contour Plot, Contour Map, Contour Histogram, or Two-Parameter Histogram)

The contourgram computes the percentage of cell groups as determined by two parameters (e.g., two monoclonal antibodies or DNA and RNA contents) (Fig. 2.7). In contrast

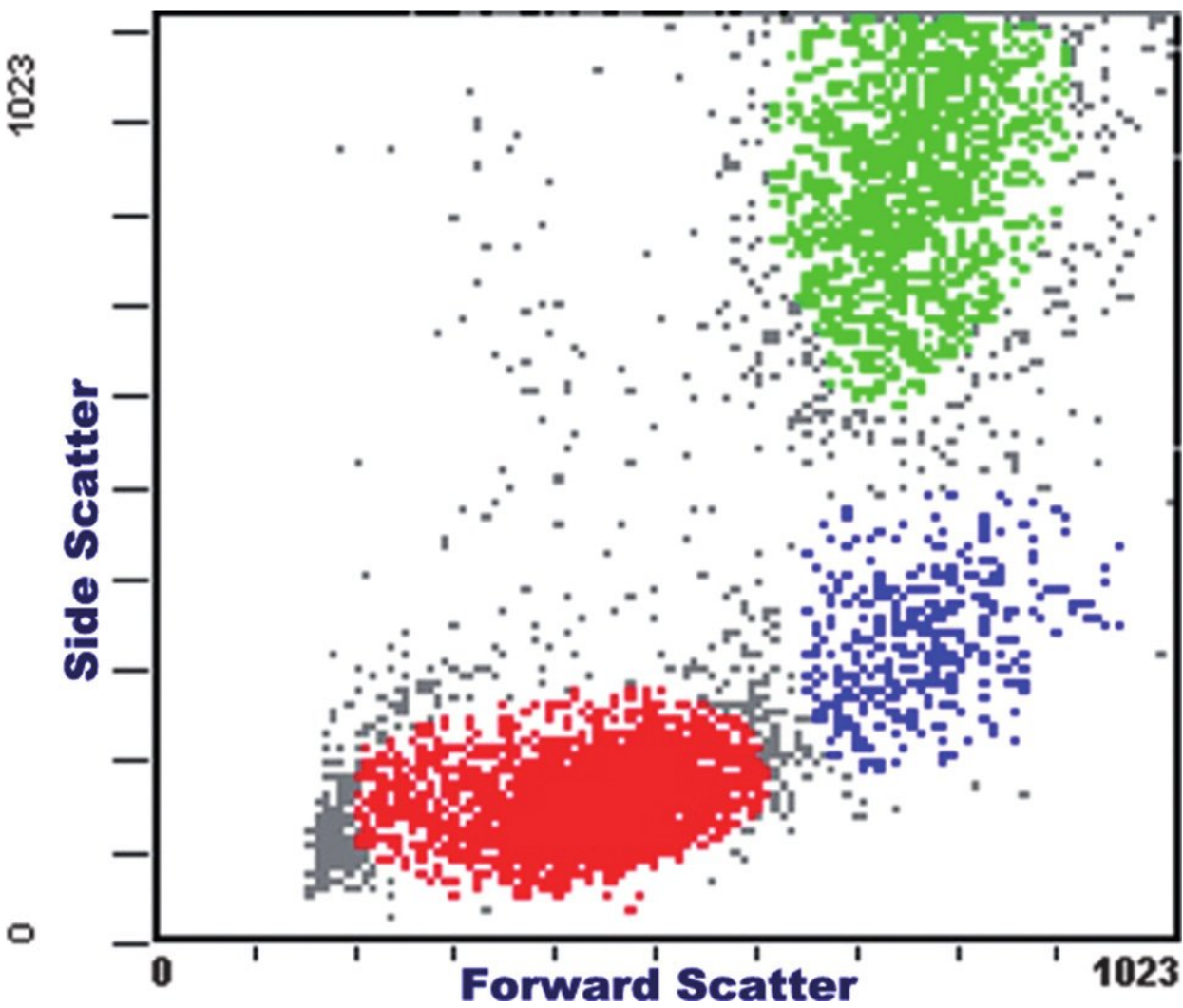


FIGURE 2.5 Scattergram with side scatter plotted against forward scatter, showing clusters of lymphocytes (red), monocytes (blue), and granulocytes (green) in a peripheral blood specimen.

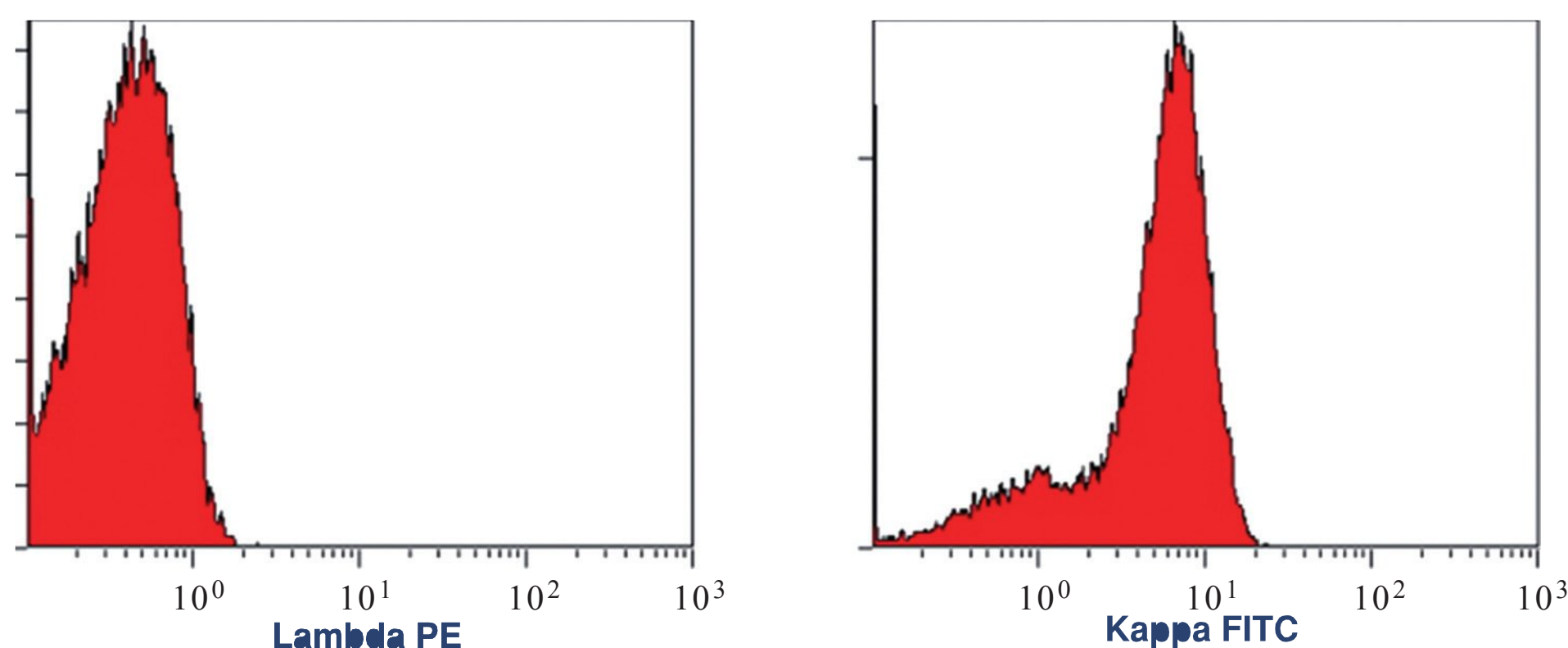


FIGURE 2.6 Histogram of a specimen stained with kappa and lambda antibodies. The lambda population is negative, whereas the kappa population is positive. PE, phycoerythrin; FITC, fluorescein isothiocyanate.

to the dot plot, the contour plot is composed of isocontour lines representing the cross sections of the peaks and valleys of the data. An isocontour line connects all elements with a similar frequency of events. When the cutoff points of these two parameters are determined, the contourgram can be divided into four quadrants, and the percentages of subpopulations are readily computed. This is a very useful means for further characterization of subpopulations. Dot plots can also be used for this two-dimensional analysis.

Isometric Plot (Three-Dimensional Isometric Curve)

The isometric plot is most frequently used for simultaneous DNA and/or RNA analysis, but it can be used to display the correlation of any three parameters (Fig. 2.8). An isometric plot is often used to present multifactor analysis

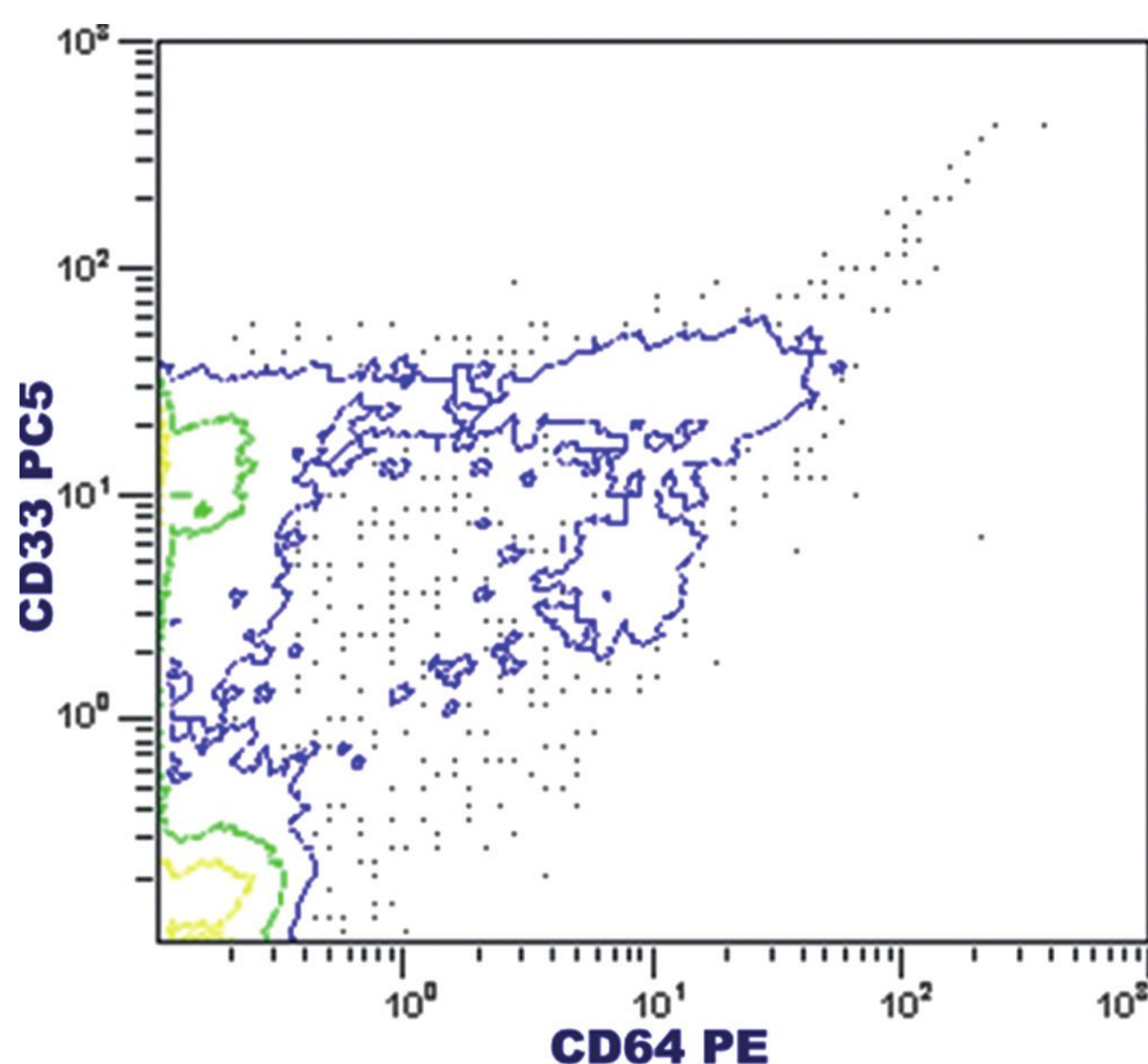


FIGURE 2.7 Contourgram of a case of acute myelomonocytic leukemia showing various populations as defined by CD33 and CD64. PC5, phycoerythrin-cyanin 5; PE, phycoerythrin.

in research projects and is seldom used in clinical laboratories for routine phenotypic analysis.

Cell Sorter

Although cell sorting can be based on the principle of electroacoustic or electromechanical fluid switching, most instruments use droplet sorting. For droplet sorting, the stream of cell suspension first passes through an ultrasonically vibrating nozzle so that the stream can be broken up into evenly spaced droplets (e.g., 30,000 to 40,000 droplets per second). At the discretion of the operator, the droplets containing the cells of interest, which have been identified by a monoclonal antibody, are selectively charged electronically, either positive or negative, and then deflected as they fall through an electromagnetic field formed by two deflection plates. The uncharged droplets fall vertically into a waste container. The cells separated in this way are usually viable and 95% pure. The cell sorter is undoubtedly a useful tool in research laboratories; however, it is not an absolute necessity for a clinical laboratory, because different groups of cells can be separated by electronic gating, and the cell group of interest can thus be selected.

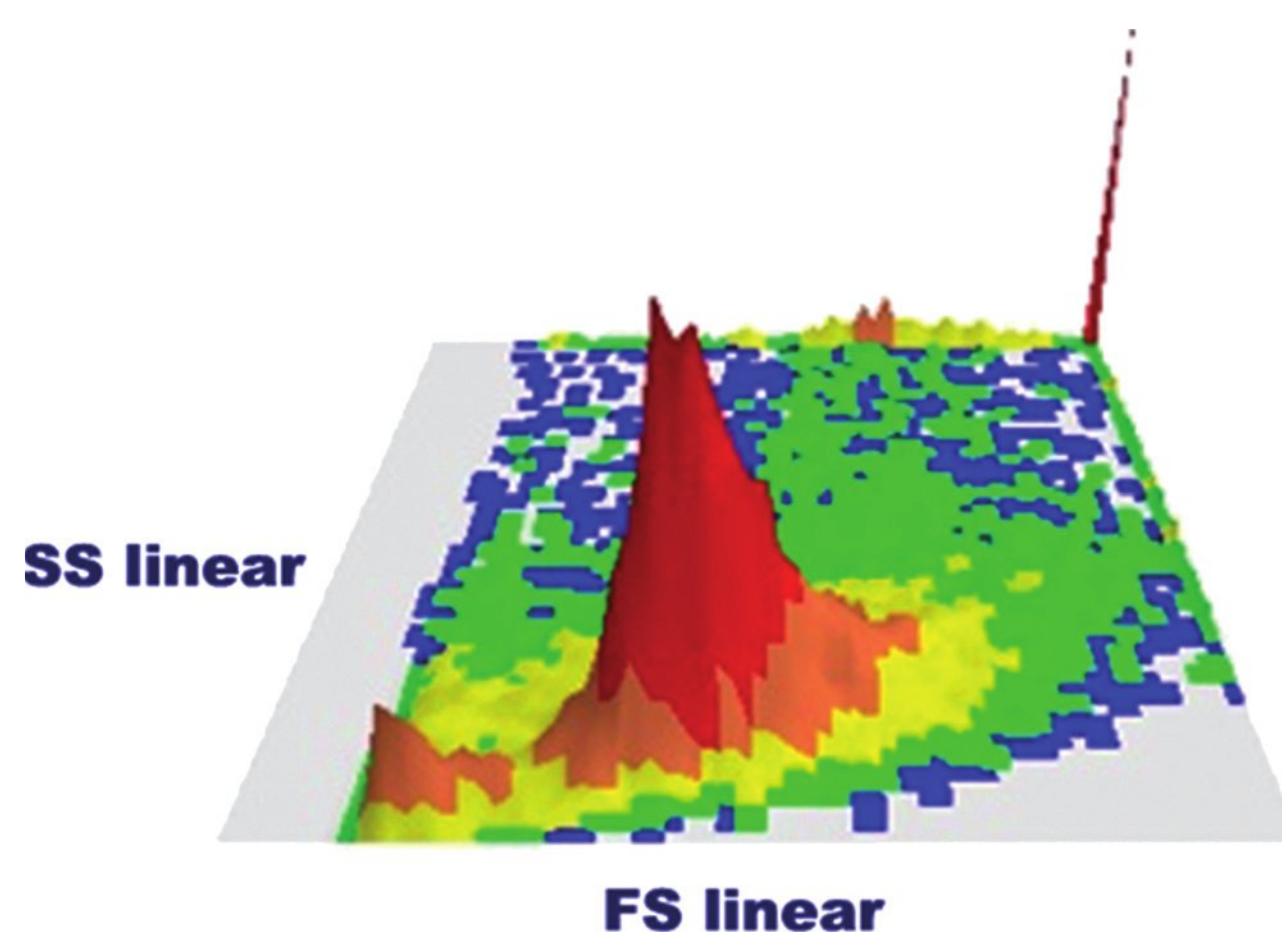


FIGURE 2.8 Isometric plot defined by side scatter (SS) and forward scatter (FS) showing a prominent peak of a leukemic population.

FACTORS AFFECTING FLOW CYTOMETRY RESULTS

A flow cytometer is a highly accurate instrument, provided that adequate quality control is maintained. For the standards and quality assurance concerning specimen collection and instrumentation in flow cytometry (FC), the reader is referred to the guidelines proposed by the National Committee for Clinical Laboratory Standards (9,10). Similar guidelines are also published by the National Institutes of Health, entitled Guidelines for Flow Cytometric Immunophenotyping (January 1993). Another excellent source of information concerning standardization and validation of laboratory procedures is the U.S.–Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by FC (11). In addition, the 2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia offers guidelines of optimal reagent and panel selection as well as quality assurance and reporting system (12).

Initial instrument setup and daily performance monitoring are the major quality assurances for the instrument (4,9,10). However, for the latest model of flow cytometers, alignment is optimized by service engineers, and daily adjustment is not required (4).

The problems affecting FC results are discussed as follows:

Specimen Problems

Besides instrumentation, the test results are also affected by inappropriate specimen collection and technical problems (Table 2.3). Recently, flow cytometrists have become increasingly aware that the patient’s condition, medication, and biologic factors may also cause some artifacts in immunophenotyping by FC (Table 2.4) (9).

From a pathologist’s point of view, the most important problem is sampling error. Samples taken from a normal area, from areas containing a small proportion of tumor cells or large numbers of degenerated or dead cells, or from specimens with necrosis or fibrosis may also give erroneous results. When a metastatic carcinoma or extensive necrosis is present in the lymph node, the scattergram may show a disarrayed dot distribution (Fig. 2.9). A splenectomy specimen should be handled promptly, because splenic tissue autolyzes rapidly due to high levels of proteolytic enzymes in the blood components.

Peripheral blood or bone marrow specimens are prepared for staining either by the lysis of erythrocytes with lysing agent, such as ammonium chloride, or by density gradient separation with Ficoll-Hypaque. These two methods for immunophenotyping are generally comparable. However, some studies found that certain populations of cells may be lost with the density gradient technique, and the consensus conference on the immunophenotyping of leukemias and lymphomas recommended this method not be used (11). In contrast, the question has been raised as to whether the lysing agent used in the whole-blood lysis may alter certain antigens (4).

One should also realize that staining intensities of tumor cells may be much more heterogeneous than staining of cells

TABLE 2.3

Factors Affecting FC Results

Sampling problems
Sampling error
Low tumor cell/normal cell ratio
Large numbers of degenerated or dead cells
Specimens with necrosis and fibrosis
Delayed processing of splenectomy specimens
Instrument problems
Improper photomultiplier adjustment for isotypic control
Improper color compensation for double labeling
Improper gating
Reagent problems
Improper antibody dilutions
Monoclonal vs. polyclonal antibodies (surface immunoglobulins)
Inappropriate fluorochrome conjugate
Miscellaneous
Cytophilic immunoglobulins
Solution: Incubation in RPMI at 37°C for 1 h
Antibody reaction to Fc receptors of tumor cells
Solution: Use Fab fragment antibody
Capping phenomenon
Solution: Use sodium azide as preservative
Phagocytosis of immunoglobulins
Solution: Eliminate monocytes

from healthy individuals because of variability in the antigens per cell (9). Even the fluorescence intensities of normal cells in abnormal specimens may show increased heterogeneity due to enhanced nonspecific binding of some (or all) antibody reagents. Therefore, there may be more overlap between positive and negative populations and more difficulty in selecting an appropriate analytic cutoff point.

Instrument Problems

To guarantee that the results obtained are meaningful, the operator of a flow cytometer should follow a step-by-step procedure. First, evaluate the viability of the cells examined with 3% Trypan blue stain; only the dead cells are stained. Viability below 80% of the cells examined may provide unreliable results. Currently, a viability dye, 7-amino-actinomycin D, is mixed with the monoclonal antibodies to demonstrate the nonviable cells in the histogram, and the viability of the cell population is automatically computed.

The second step is gating to select the cell cluster for analysis. For peripheral blood specimens, forward scatter versus side scatter is still the routine strategy. This plot provides the best visual differentiation between lymphocytes, monocytes, and granulocytes (13). For lymphoma or lymphoid leukemias, the cluster of lymphocytes should

TABLE 2.4

Potential Sources of Artifacts in Immunophenotyping by FC

Cause	Effect	Resulting artifact
Medications of drugs		
Zidovudine (AZT)	Increased granulocyte fragility	Decreased light on scatter resolution, increased granulocyte contamination of mononuclear preparation
Some antibiotics (e.g., cephalosporins)	Increased cellular autofluorescence	False positive if appropriate negative control is not used
Some chemotherapeutic agents (e.g., daunorubicin)	Increased cellular autofluorescence	False positive if appropriate negative control is not used
Nicotine	Increased lymphocyte margination, decreased lymphocyte count	Lower absolute values of lymphocyte subsets
Corticosteroids	Decreased CD4 levels	Overestimation of disease-related alterations
Antihuman lymphocyte antibodies (e.g., CD3 or soluble CD4)	Lymphocytopenia, modulation, or blocking of cell surface receptors	Decreased labeling with antibody reagent
Biologic factors		
Reticulocytosis	Incomplete red cell lysis, increased contamination of mononuclear preparations	Decreased light scatter resolution, red blood cell contamination of lymphocyte gates
Strenuous exercise	Increased lymphocyte margination, decreased lymphocyte counts	Lowered absolute values for lymphocyte subsets
Diurnal variation	Variable absolute lymphocyte count	Variable absolute subset values
Specimen age and holding condition	Variable granulocyte preservation and/or leukocyte viability	Increased granulocyte contamination of lymphocyte gates, false-positive nonspecific staining of dead cells

National Committee for Clinical Laboratory Standards. Clinical Application of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes. 2nd ed. (H42-A2). Wayne, PA: National Committee for Clinical Laboratory Standards; 2006.

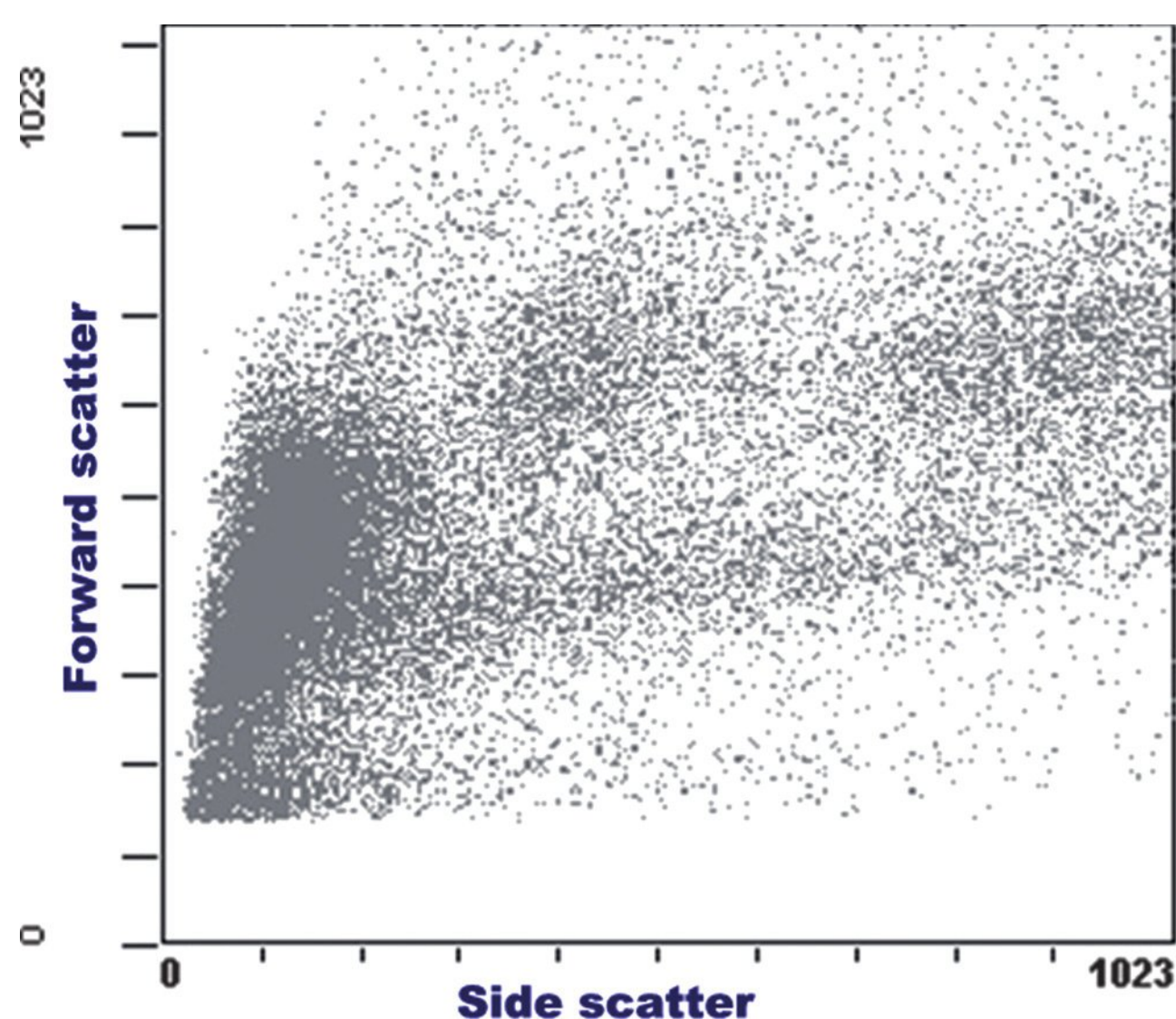


FIGURE 2.9 Scattergram of a lymph node biopsy with extensive necrosis showing a disarrayed dot distribution.

be selected electronically for analysis. As will be discussed later, other gating strategies may be considered for specimens other than blood. The third step is back-gating to check the correctness of the gated population. The combination of this plot is CD45, the panleukocyte marker also known as leukocyte common antigen, versus CD14, a monocyte marker (14). As a result, the monocytes and more differentiated hematopoietic precursor cells are represented in quadrant 2 of a two-parameter histogram, as they react to both CD14 and CD45 (15). In quadrant 4, two groups of cells can be identified because they react only to CD45. The CD45-bright group represents mainly lymphocytes and the CD45-dim group, neutrophils (15). Lymphoblasts and myeloblasts are CD45 dim or CD45 negative. When the lymphocyte cluster is further separated into large and small lymphocyte groups, this can be checked by a single histogram of forward scatter to see if two peaks are clearly separated (Fig. 2.10).

Gating

Gating is the most important step in making a correct diagnosis. Many problematic cases are either the result of

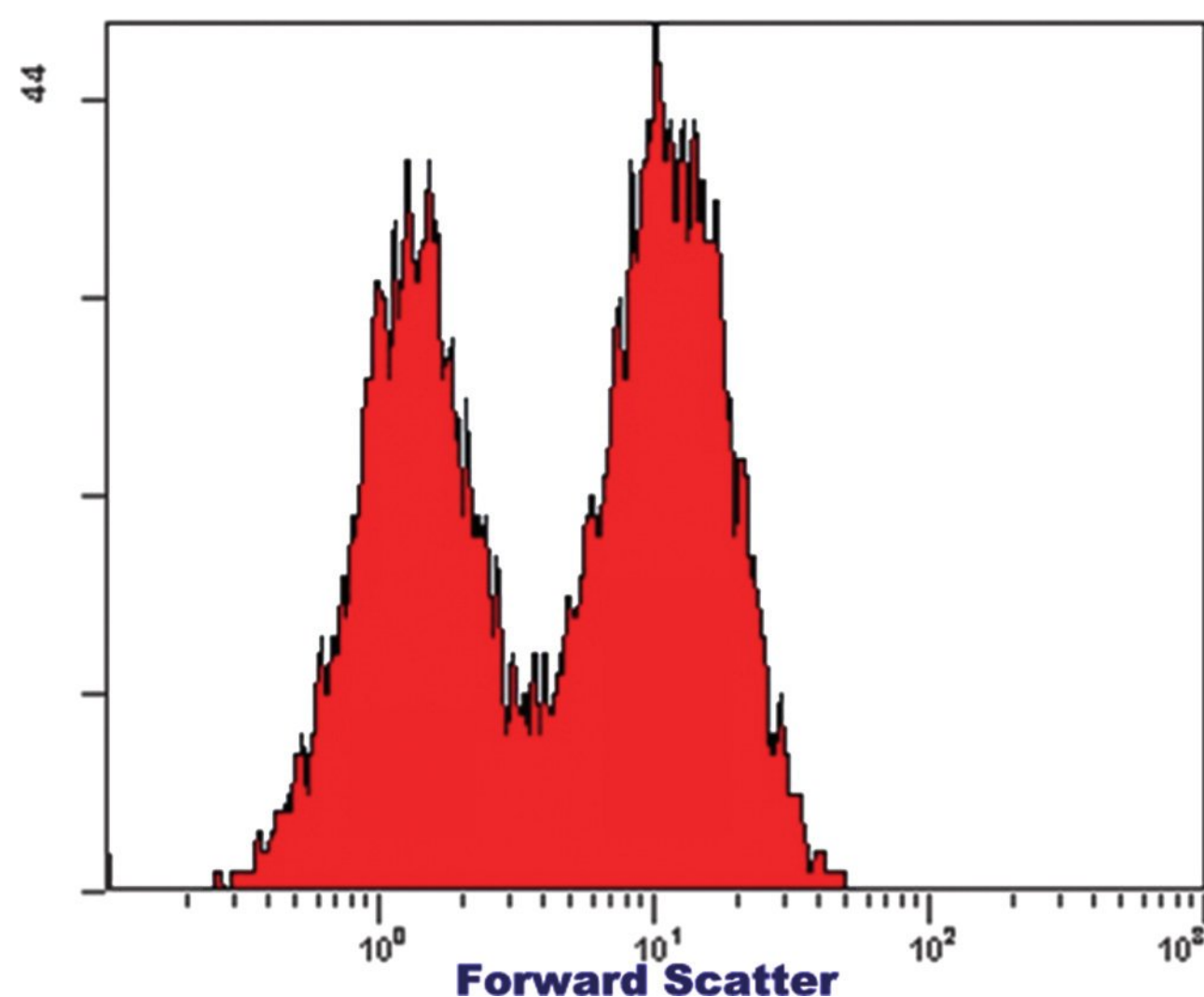


FIGURE 2.10 Histogram of forward light scatter showing small- and large-cell populations from a lymphocyte gate.

“loose” gating (e.g., a big lymphocyte gate including other cell populations) or “tight” gating (only the central part of the cell cluster is gated). A loose gating will result in a lower percentage of the target population. In contrast, tight gating may inadvertently eliminate some B cells and natural killer cells, which have lower and higher forward scatter, respectively, than do T cells.

In addition to this homogeneous gating based on physical properties of cells (light scatter), there is also heterogeneous gating by using fluorescence antibody versus side scatter. The T-gating method used CD3 as a T-cell marker (16), B gating uses CD19 (17), and leukocyte gating uses CD45 (18). The T-gating method should be more specific than the routine homogeneous gating for T-cell subset analysis.

In specimens other than peripheral blood, there is an excess of nonleukocytes exhibiting leukocyte-like scatter characteristics leading to a low fraction of recovered leukocytes (19). Furthermore, even in the leukocyte populations, the cell size and cytoplasmic complexity may overlap, causing some difficulty in their separation (20). Substantial inaccuracy may occur when this gated population is analyzed after specific monoclonal antibody staining (15). This is especially true when the light scatter gate is used to identify a leukemic population (21).

A new gating strategy was first advocated by Stelzer et al. (20), who used a combination of right-angle light scatter and CD45 (panleukocyte antibody) for flow cytometric analysis of normal bone marrow specimens. Subsequently Festin et al. (19) applied the same combination to enumerate lymphoid cells in posttransplant bone marrow and liver biopsy specimens. Borowitz et al. (21) and Rainer et al. (22) used this gating strategy for immunophenotyping of acute leukemia in bone marrow. Nicholson et al. (23) applied the same principle to T-cell subset studies in whole-blood specimens. In our study, we found that this gating strategy is not only suitable for acute leukemia

specimens in the bone marrow but can also be applied to the study of chronic leukemias and lymphomas in the peripheral blood, bone marrow, lymph node, or other soft tissues (24). When comparing this new gating technique with conventional gating, we found that the percentage of tumor cells isolated by the new gating is consistently higher than that isolated by the conventional one, no matter whether it is lymphoma or leukemia or what kind of specimen is involved.

Cursor Setting

The second important step is setting the cutoff point (cursor setting) between positive cells and negative cells. The negative cells are the cells from the same patient incubated with mouse immunoglobulin of the same isotype as the monoclonal antibody used for the study (isotypic control). However, cursor setting is sometimes complicated, and certain adjustment with the photomultiplier tube is needed. If the photomultiplier tube is underadjusted, the weakly positive peak may become false negative. In contrast, overadjustment may put the strongly positive peak off the screen.

As the isotypic control is sometimes superfluous and potentially misleading, many authors advocate pattern reading rather than presentation of percentages of various populations (4,11). It is certainly not appropriate to depend blindly on isotypic controls to distinguish positive and negative populations, but quantitative report is helpful for therapeutic monitoring and should be retained. In case of doubt, a contourgram is sometimes useful to identify the dim population, which can be mistaken as partial negative (Fig. 2.11). Report in a simple tabular form of percentages of all markers is discouraged in the 2006 Bethesda conference, but the reporting of the percent of abnormal cells present in the specimen is required (12). In recent years, many innovative ways of data analysis have been proposed, including the SPIC software, probability binning, frequency difference gating, cluster analysis, and principal component analysis (25).

Electronic Compensation

In double labeling, two fluorochromes, such as fluorescein and PE, are used. Although both fluorochromes are excited at 488 nm, they emit at different wavelengths. However, the spectra of these emissions overlap. Therefore, electronic adjustment (a procedure known as electronic compensation) must be made to correct spectral overlap. Undercompensation may sometimes cause false-positive results showing a double-labeled population. Overcompensation, in contrast, causes false-negative results as the positive populations may be off the screen.

Reagent Problems

The major reagent problems include improper antibody dilution, selection of antibody, and conjugation of fluorochrome. Monoclonal antibodies are generally used for FC except for the study of surface immunoglobulins. Immunoglobulins have many epitopes; therefore, polyclonal antibodies are more likely to react positively than

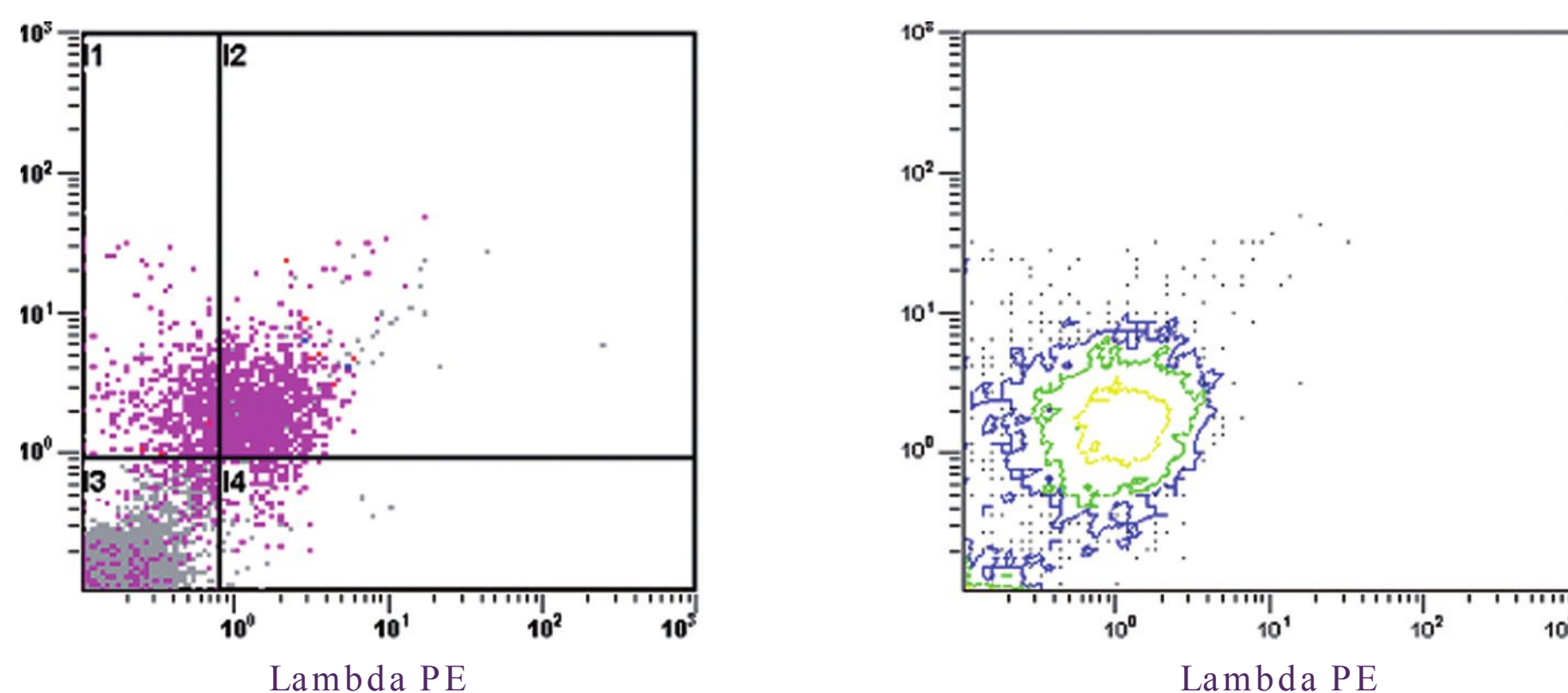


FIGURE 2.11 Scattergram plotted by CD20 versus lambda (**left**). The dividers as determined by isotopic controls incorrectly define the positive population. Contourgram (**right**) correctly identifies an intact cluster of chronic lymphocytic leukemia cells that have dim staining in both CD20 and lambda. PC5, phycoerythrin–cyanin 5; PE, phycoerythrin.

are monoclonal antibodies. A neoplasm may not show a certain epitope of the immunoglobulin that is specific for a monoclonal antibody. For instance, the antibody may be specific for IgG-1 subclass, whereas the tumor cells may express IgG-4 subclass.

FITC has a low quantum yield, and its spectrum overlaps with autofluorescence of lymphocytes (16,17). Therefore, FITC should be conjugated with an antibody that reacts to a high-density antigen on the cell surface, such as CD45 or human leukocyte antigen (HLA-DR). In contrast, weak antigens, such as CD13, CD10, and CD33, should be conjugated with bright fluorochromes (5). The fluorochrome intensity is brightest among PE and its tandem fluorochromes including PE-Texas Red, PE-cyanin 5 (PE-Cy5), PE-Cy5.5, and PE-Cy7 (24). This is followed by the allophycocyanin (APC) group including APC, APC-Alexa 700, and APC-CY7. The small-molecule fluorochromes, including FITC, peridinin chlorophyll protein, Pacific Blue, and the Alexa series of dyes, exhibit fluorescence approximately 1 log dimmer than the above fluorochromes (26).

Miscellaneous

Other potential problems can be avoided by using standard procedures (9). For instance, cytophilic immunoglobulin (patient's own serum immunoglobulin that adheres to lymphocyte surface) may cause aberrant results by reacting with anti-immunoglobulin antibodies. The cytophilic immunoglobulin can be eliminated by washing after the lymphocytes are incubated in phosphate-buffered saline or RPMI medium at 37°C for 1 hour.

The Fc receptor is present on the surface of lymphocytes and monocytes. If the antibody used contains the Fc fragment, false-positive results may be obtained. Because it is now common practice to use antibodies with an Fab fragment, this nonspecific reaction is no longer a problem.

A capping phenomenon is frequently seen in surface immunoglobulin reaction, because the anti-immunoglobulin–surface immunoglobulin immune complex can be internalized, leaving only a small portion of the complex on the cell surface (capping). This may lead

to a false-negative result. The routine use of sodium azide as a preservative for antibody reagents and as an additive to phosphate-buffered saline diluent may prevent this phenomenon.

Finally, phagocytosis of immunoglobulin by monocytes or macrophages may give rise to false-positive results for surface immunoglobulin. Pretreatment to separate the phagocytic cells from lymphocytes and careful gating to eliminate monocyte contamination of the lymphocyte gate are measures to prevent this phenomenon.

In summary, many factors can affect the flow cytometric results. Therefore, strict adherence to standard procedure and to quality assurance is mandatory for obtaining clinically meaningful results.

PARAMETERS FOR CELL DISTINCTION

After ensuring that the instrument, sampling, and reagent problems are under control, one can start to analyze patients' specimens. The major drawback for FC is lack of correlation between morphology and cell markers. Therefore, many parameters are being used to characterize different cell types. The parameters that help to distinguish different cell populations are listed in Table 2.5.

Cell Size

As mentioned before, cell size can be determined by forward scatter. Cell size is not only important in distinguishing normal leukocytes (lymphocyte vs. monocyte vs. granulocyte) but it can also differentiate large tumor cells from small reactive lymphocytes and a mixed large and small lymphoma cell population.

Cytoplasmic Granularity

Cytoplasmic granularity can be determined by side scatter. This is the second important parameter. Cell size combined with cytoplasmic granularity can distinguish the three major types of leukocytes in the blood.

TABLE 2.5

Parameters for Cell Distinction by FC

1. Cell size
2. Cytoplasmic granularity
3. Immunophenotyping: identify cell lineage, developmental stages, and clonality
4. Relative percentage of reactive cells: kappa/lambda ratio, selective loss of pan-T-cell markers
5. Intensity of surface immunoglobulin: dim immunofluorescence on CLL cells
6. Double labeling: CD19/CD5 for CLL/mantle cell lymphoma, CD22/CD11c for hairy cell leukemia
7. DNA/RNA contents: diploidy vs. aneuploidy, increased RNA in proliferating cells

CLL, chronic lymphocytic leukemia.

Immunophenotyping

Immunophenotyping is the use of monoclonal antibodies to identify the surface, cytoplasmic, and nuclear antigens in individual cells, thus constructing a phenotype of these cells based on positive and negative reactions to a group of antibodies. For instance, detection of both a monoclonal B-cell population and a polyclonal T-cell population in

a lymph node is consistent with a B-cell lymphoma with reactive T lymphocytes. With five- to eight-color FC, a very accurate immunophenotyping can be achieved (Fig. 2.12).

Comparison of Percentages of Reactive Cells

This is an important parameter used to draw a preliminary conclusion. For instance, either kappa light chain or lambda light chain antibodies can be used to identify B cells, but when the kappa/lambda ratio is calculated, it can determine the clonality of a B-cell population. By the same token, a prominent discrepancy between the percentage of different pan-T-cell markers (CD3, CD5, and CD7) is suggestive of T-cell neoplasms (27).

Intensity of Immunofluorescence of Surface Immunoglobulin

This correlates with the amount of immunoglobulin present on the surface of B cells. As a rule, the follicular center cells stain more brightly than do medullary cord cells (28). Thus cells from follicular lymphoma stain brightly, and those from chronic lymphocytic leukemia stain dimly. Chronic lymphocytic leukemia transforming into a large-cell lymphoma (Richter syndrome) may be detected by FC on the basis of the presence of two patterns of immunofluorescence intensity and immunophenotypes (29).

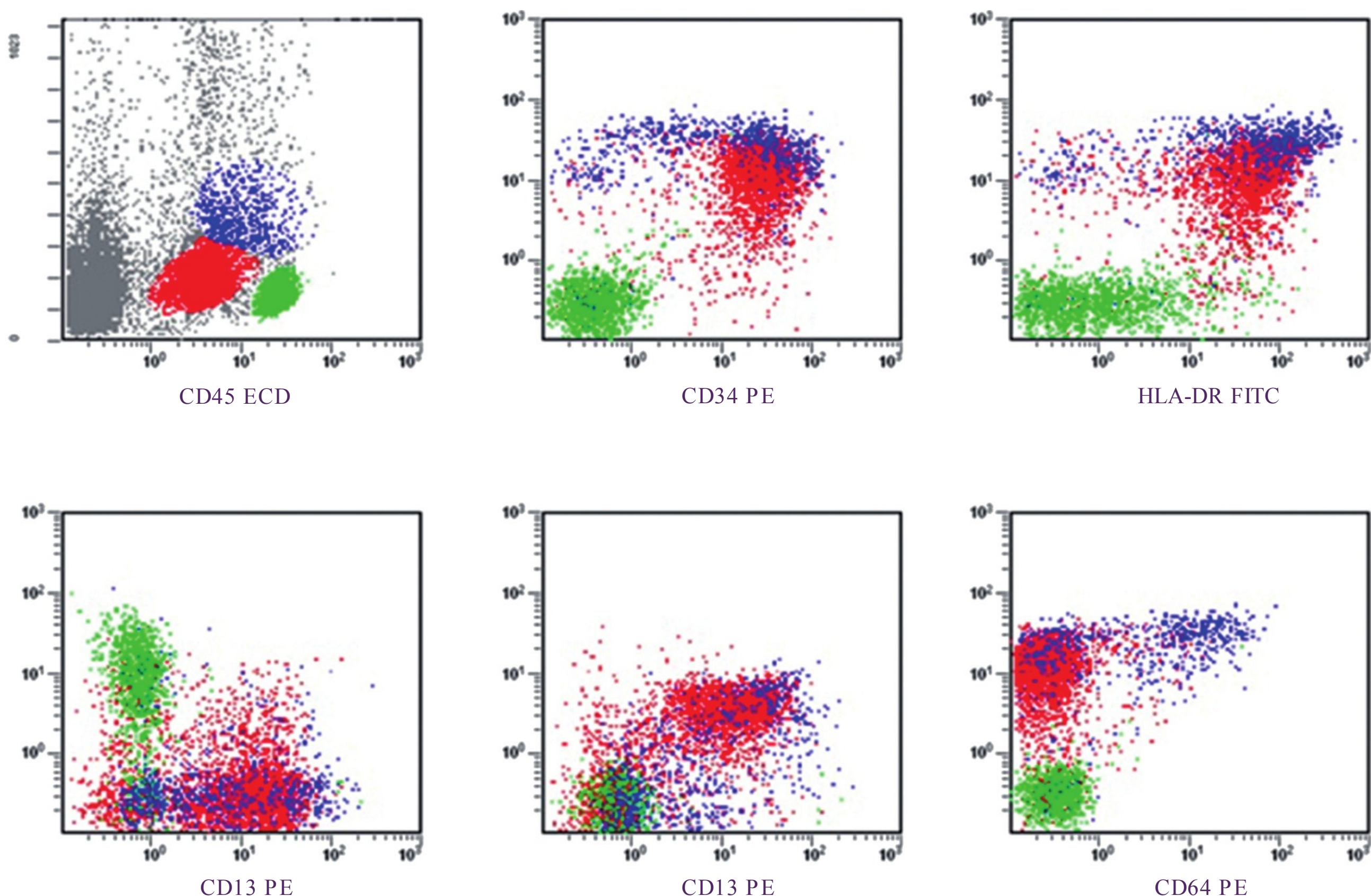


FIGURE 2.12 Five-color FC demonstrates three cell populations in a case of acute myelomonocytic leukemia (myelocytes, red; monocytes, blue; lymphocytes, green).

Double Labeling

This is accomplished by the two-color analysis setting in flow cytometers. Whereas single-marker labeling may identify the lineage of certain cells, double labeling may define a neoplastic population. The most common example is the coexistence of CD19 and/or CD20 (B-cell markers) with CD5 (T-cell marker) in chronic lymphocytic leukemia and/or small lymphocytic lymphoma as well as mantle cell lymphoma (30). Another example is hairy cell leukemia, which expresses both a B cell (CD22) and a monocyte marker (CD11c) (31). With the availability of six-color analysis, more accurate and more defined tumor population detection will be achieved.

DNA and RNA Contents

The DNA/RNA ratio may differ between a tumor cell and a normal cell population. For instance, tumor cells may show an aneuploid peak, whereas normal cells express a diploid peak. The increase in RNA content in the cell cycle is a good indicator of cell growth.

CONCLUSION

Over the last decade, FC has made tremendous progress due to the improvement of instrumentation and addition of well-defined monoclonal antibodies, many of which are derived from the newly discovered gene products. As a result, FC is able not only to diagnose a wide spectrum of lymphomas and leukemias with high accuracy but also to monitor treatment and predict prognosis reliably. Some FC immunophenotypes correlate well with certain karyotypes, thus helping to stratify the risk groups for clinicians to tailor the treatment. The recently established phosphoflow cytometry technique opens an exciting path for functional studies of pathogenic signaling profile. Phosphorylation is the major mechanism for intracellular signaling. Therefore, the simultaneous immunologic measurement of cell lineage antigens and phosphorylated epitopes of signaling proteins before and after the treatment with a specific modulator can reveal changes of the signaling profile of tumor cells (32). These results provide important information relating to tumorigenesis, which can be useful for targeting inhibition of certain oncogenes. Another new flow technology called ImageStream is able to detect the cell markers and digital image of tumor cells at the same time, providing direct correlation between immunology and morphology (33). It appears that there are unlimited potentials in the application of FC technology, which will undoubtedly become tremendously valuable in our evaluation of hematologic neoplasms.

REFERENCES

1. Grogan WM, Collins JM. *Guide to Flow Cytometry Methods*. New York: Marcel Dekker; 1990:1–21.
2. Leith CP, Willman CL. Flow cytometric analysis of hematologic specimens. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:255–270.
3. McCoy JP Jr. Basic principles in clinical flow cytometry. In: Keren DF, McCoy JP Jr, Carey JL, eds. *Flow Cytometry in Clinical Diagnosis*. 3rd ed. Chicago, IL: ASCP Press; 2001:31–64.
4. McCoy JP Jr. Flow cytometry. In: McClatchey KD, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:1401–1425.
5. Riley RS. Structural components of the flow cytometer. In: Riley RS, Mahin EJ, William R, eds. *Clinical Application of Flow Cytometry*. New York: Igaku-Shoin; 1993:17–60.
6. Shapiro HM. *Practical Flow Cytometry*. 4th ed. Hoboken, NJ: John Wiley & Sons; 2003:1–60.
7. Wheelless LL Jr. Flow instrumentation and data analysis. In: Coon JS, Weinstein RS, eds. *Diagnostic Flow Cytometry*. Baltimore, MD: Williams & Wilkins; 1991:17–34.
8. Wood JCS. Clinical flow cytometry instrumentation. In: Bauer KD, Duque RE, Shankey TV, eds. *Clinical Flow Cytometry: Principle and Application*. Baltimore, MD: Williams & Wilkins; 1993:71–92.
9. National Committee for Clinical Laboratory Standards. *Clinical Application of Flow Cytometry: Quality Assurance and Immunophenotyping of Peripheral Blood Lymphocytes*. 2nd ed. (H42-A2). Wayne, PA: National Committee for Clinical Laboratory Standards; 2006.
10. National Committee for Clinical Laboratory Standards. *Clinical Application of Flow Cytometric Immunophenotyping of Leukemic Cells: Proposed Guideline: Clinical Flow Cytometric Analysis of Neoplastic Hematolymphoid Cells*. 2nd ed. (H43-A2). Wayne, PA: National Committee for Clinical Laboratory Standards; 2006.
11. Stelzer GT, Marti G, Hurley A, et al. U.S.-Canadian consensus recommendation on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: standardization and validation of laboratory procedures. *Cytometry*. 1997;30:214–230.
12. Wood BL, Arroz M, Barnett D, et al. 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow cytometry: Optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry Part B*. 2007;728:S14–S22.
13. Saltzman GC, Crowell JM, Martin JC, et al. Cell classification by laser light scattering: identification and separation of unstained leukocytes. *Acta Cytol*. 1975;19:374–377.
14. Rothe G, Schmitz G, Adort D, et al. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877–895.
15. Loken MR, Brosman JM, Bach BA, et al. Quality control in flow cytometry. 1. Establishing optimal lymphocyte gates for immunophenotyping by flow cytometry. *Cytometry*. 1990;11:453–459.
16. Shapiro HM. Quantitative immunofluorescence measurements and standards. Practical approaches. *Clin Immunol News*. 1992;11:49–54.
17. Mandy FF, Bergeron M, Izaguirre CAL. Application tools for clinical flow cytometry: gating parameters for immunophenotyping. *Clin Immunol News*. 1992;12:25–32.
18. Parker JW. Immunologic basis for the redefinition of malignant lymphomas. *Am J Clin Pathol*. 1979;12(suppl):670–686.
19. Festin R, Bjorkland A, Totterman TH. Multicolor flow cytometric analysis of the CD45 antigen provides improved lymphoid cell discrimination in bone marrow and tissue biopsies. *J Immunol Methods*. 1994;177:215–224.
20. Stelzer GT, Shuts KE, Loken MR. CD45 gating for routine flow cytometric analysis of human bone marrow specimens. *Ann N Y Acad Sci*. 1993;677:265–280.



21. Borowitz MJ, Guenther KL, Shults KE, et al. Immunophenotyping of acute leukemia by flow cytometric analysis use of CD45 and right-angle light scatter to gate on leukemic blasts in three color analysis. *Am J Clin Pathol*. 1993;100:534–540.
22. Rainer RO, Hodges L, Stelzer GR. CD45 gating correlates with bone marrow differential. *Cytometry*. 1995;22:139–145.
23. Nicholson JKA, Jones BM, Hubbard M. CD4 T-lymphocyte determinations on whole blood specimens using a single-tube three color assay. *Cytometry*. 1993;14:685–689.
24. Sun T, Sangaline R, Ryder J, et al. Gating strategy for immunophenotyping of leukemia and lymphoma. *Am J Clin Pathol*. 1997;108:152–157.
25. Lugli E, Roederer M, Cossarizza A. Data analysis in flow cytometry: the future just started. *Cytometry Part A*. 2010;77A:705–713.
26. Wood B. 9-color and 10-color flow cytometry in the clinical laboratory. *Arch Pathol Lab Med*. 2006;130:680–690.
27. Sun T, Ngu M, Henshall J, et al. Marker discrepancy as a diagnostic criterion for lymphoid neoplasms. *Diag Clin Immunol*. 1988;5:393–399.
28. Aisenberg AC. Cell surface markers in lymphoproliferative disease. *N Engl J Med*. 1981;304:331–336.
29. Sun T, Susin M, Desner M, et al. The clinical origin of two cell populations in Richter's syndrome. *Hum Pathol*. 1990;21:722–728.
30. Weisenburger DD, Duggan MJ, Perry DA, et al. Non-Hodgkin's lymphoma of mantle zone origin. *Pathol Ann*. 1991;26(pt 1):139–158.
31. Schwarting R, Stein H, Wang CY. The monoclonal antibodies aS-HCL1 (aLeu-14) and aS-HCL3 (a Leu-M5) allow the diagnosis of hairy cell leukemia. *Blood*. 1985;65:974–983.
32. Covey TM, Cesano A. Modulated multiparametric phosphoflow cytometry in hematological malignancies: technology and clinical applications. *Best Prac Res Clin Hematol*. 2010;23:319–331.
33. de Tute RM. Flow cytometry and its use in the diagnosis and management of mature lymphoid malignancies. *Histopathology*. 2011;58:90–105.

3

Principles of Immunohistochemistry

For many years, pathologists had depended on morphology alone to make a histologic diagnosis until the availability of “special stains.” Special stains were developed because pathologists always realized the fallacy of the morphologic approach and felt the need to have some accessory tests to substantiate the diagnosis. The first kind of special stains are cytochemical stains, which mainly identify cell lineage and cellular chemistry. When cytochemical techniques are applied to histologic sections, it is called histochemistry, which further improves the accuracy of a morphologic diagnosis. However, it is the development of immunohistochemistry that finally makes histologic diagnosis highly objective. Immunohistochemistry is particularly indispensable for the practice of hematopathology, but the molecular cytogenetic techniques have also played an increasingly important role in the diagnosis of hematologic neoplasms. The advent of nonradioactive in situ hybridization (NISH) techniques represents the current effort to combine histologic staining with the studies of nucleic acids (DNA and RNA). These various entities are discussed briefly in this chapter.

CYTOCHEMISTRY

Cytochemistry is an integral part in the diagnosis of acute myeloid leukemia (AML) required by the French-American-British Cooperative Group (1,2). Although its role has been gradually replaced by immunophenotyping with flow cytometry, cytochemistry is still useful in identifying cell lineages, especially the immature monocytes in the bone marrow, which are frequently difficult to recognize morphologically.

Myeloperoxidase (MPO) is the first screening test to distinguish AML from acute lymphoblastic leukemia (ALL). This enzyme is present in neutrophilic, eosinophilic, and monocytic lineage but not in lymphocytes (3). However, the minimally differentiated myeloblasts may not stain for MPO as seen in the AML-M0 cases, which can be distinguished from ALL only by immunophenotyping. The MPO in eosinophils is resistant to cyanide, so that eosinophil and its immature forms can

be identified by this specific reaction. The peroxidase of megakaryocytes and platelets cannot be visualized by light microscopy, but it can be demonstrated by electron microscopy.

Sudan black B reaction is slightly more sensitive than, but similar to, MPO reactions. Sudan black B is a fat-soluble substance, but it probably stains for substances related to MPO, because the Sudan black B reaction becomes negative in patients with MPO deficiency (4). In addition to the MPO-positive cells, Sudan black B also stains fat cells, macrophages, and cytoplasmic vacuoles in Burkitt lymphoma cells.

Specific esterases are a group of enzymes capable of hydrolyzing halogenated naphthol esters (3). The most commonly used substrate is naphthol AS-D chloroacetate. Chloroacetate esterase is most frequently used for the identification of neutrophilic series and occasionally used for mast cells. Because chloroacetate esterase is stable even in paraffin-embedded tissue, it can be used for the diagnosis of myeloid sarcoma and extramedullary hematopoiesis. Chloroacetate esterase is negative for monocytes, megakaryocytes, erythroblasts, and lymphocytes. Only abnormal eosinophils, such as those seen in AML with bone marrow eosinophilia, are positive for chloroacetate esterase.

Nonspecific esterases are a group of enzymes capable of hydrolyzing various aliphatic and aromatic short-chain esters (3). They are called nonspecific esterases because these enzymes exhibit a wide range of substrate specificity. The substrates used to detect nonspecific esterase activity include α -naphthyl butyrate, α -naphthyl acetate, naphthol AS-D acetate, and naphthol AS acetate. The first two substrates are most frequently used because they do not stain for granulocytes. Thus, sodium fluoride inhibition is not needed to distinguish monocytes from granulocytes. Besides monocytes and histiocytes, α -naphthyl acetate esterase is also positive in megakaryocytes and platelets, and the reaction is sodium fluoride sensitive (4). T lymphocytes usually show a focal paranuclear staining for nonspecific esterases. Because nonspecific esterases are sensitive to heat, storage, and fixative, they cannot be demonstrated in paraffin-embedded tissue sections.

Acid phosphatases (APs) are a group of enzymes capable of hydrolyzing monophosphate esters in an acid environment (3). By electrophoresis, APs can be separated into seven nonerythrocytic isoenzymes. Isoenzymes 2 and 4 are present in neutrophils and monocytes; 3, in lymphocytes and platelets; 3b, in primitive blood cells and blasts; and 5, in hairy cells of hairy cell leukemia. The most important function of AP is to identify hairy cells, which show a strong, diffuse, tartrate-resistant AP. This reaction may be demonstrated occasionally in other lymphomas or leukemias, but the reaction is seldom as intense and diffuse as in hairy cell leukemia. The focal paranuclear staining pattern of AP is helpful in identifying T lymphocytes.

Periodic acid-Schiff (PAS) is capable of reacting with R-CHO groups in tissues to form an insoluble bright-red complex (aldehyde–fuchsin–sulfurous acid compound) (5). Therefore, tissue and cells containing glycoproteins, mucoproteins, and high-molecular-weight carbohydrates are positive for PAS. The PAS reaction is positive in most blood cells; it is detected in 80% to 90% of cases of ALL and in 10% to 15% of cases of AML (3). It is particularly useful when a block pattern is demonstrated in pronormoblasts and lymphoblasts. A positive PAS stain in erythroblasts is a common finding in erythroleukemia; it is a coarsely granular pattern in cells of early stage and a finely granular pattern in cells of later stage. Normoblasts in healthy persons are PAS negative, but they can be PAS positive in erythrodysplasia. PAS staining of the periphery of cytoplasm, especially in cytoplasmic protrusions, is characteristic for megakaryocytes and megakaryoblasts. This pattern, if present, is helpful for the diagnosis of acute megakaryoblastic leukemia.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry is the application of a labeled or enzyme-bound antibody to identify a specific antigen, which is visualized under light microscopy by means of a color signal. There are several important milestones in the history of immunohistochemistry development (5). In 1940, Coon first used immunofluorescence techniques to detect antigens in frozen sections (6). Avrameas (7) developed enzymatic labeling to demonstrate the antigen–antibody immune complex in tissue sections. Taylor and Burns (8) first applied immunohistochemical techniques to formalin-fixed, paraffin-embedded tissue sections. The subsequent progression from the one-step direct conjugate method to the multiple-step indirect method as well as to the discovery of the hybridoma technique by Kohler and Milstein (9) greatly facilitated the versatility of immunohistochemistry. The staining technique is further enhanced by enzyme digestion (10) and finally by antigen retrieval (AR) techniques (11,12) that make immunohistochemistry the indispensable tool in the practice of surgical pathologists. The current goal is to make immunohistochemistry quantitative so that it is a tool not only for diagnosis but also for therapeutic monitoring and prediction of prognosis in various tumors (13,14).

Immunohistochemical staining involves multiple and somewhat complicated steps; therefore, there are many technical problems to watch for, and there are many technical decisions to make depending on the target antigens. Due to space limitation, only a few important technical matters are discussed in this chapter. For a comprehensive review, the reader is referred to the textbooks by Dabbs (15) and Elias (16).

Fixatives

There are two groups of fixative: the cross-linking fixatives (e.g., formaldehyde) and coagulant or precipitation fixatives. The latter group includes acid fixatives (e.g., Bouin solution) and heavy metal fixatives (B5 and Zenker fluid). Williams et al. (17) compared the effects of various fixatives in immunohistochemical stain results on tonsil tissue and found that 10% neutral buffered formalin (NBF), 10% zinc formalin, and 10% formal saline gave the most consistent results overall and showed excellent antigen preservation. In contrast, 10% formal acetic acid, B5, and Bouin fixative showed poor antigen preservation.

Others feel that there is no particular optimal fixative because the staining results depend on a complex interaction among the fixative, pH, osmolarity, temperature, length of treatment, and tissue types (16). For instance, there is no difference between formalin and Bouin fixative for the staining of insulin, pancreatic polypeptide, and gastrin, but better results are obtained by Bouin fixative than by formalin for the staining of glucagons and somatostatin (18). Elias (16) suggested dividing specimens into multiple fixatives, including NBF, 10% formal saline, 95% ethanol, Omni, modified methacarn, and B5 for subsequent processing. B5 is considered most suitable for fixation of lymphoid tissues but, because it contains mercury, many laboratories avoid using it.

Immunohistochemical Staining Procedures

Direct Conjugate-Labeled Antibody Method

The direct method is the application of labeled monospecific antibody directly to the tissue section (Fig. 3.1). The label can be an enzyme, biotin, fluorochrome, or colloidal gold. There are several procedures that are used to enhance the direct method. The most common one is the use of biotin to conjugate the antibody, and then the biotin will bind to the receptor of either the labeled avidin or streptavidin. With the second layer of labeling, the staining becomes amplified.

Indirect or Sandwich Method

The indirect method includes two major procedures: the indirect labeled procedure and the indirect unlabeled antibody procedure. The first procedure uses two layers of antibodies; the unlabeled primary antibody directly reacts to the tissue antigen, and the labeled secondary antibody reacts to the primary antibody (Fig. 3.2). The unlabeled antibody procedure uses three layers of antibodies (Fig. 3.3). The tertiary antibody is an antienzyme antibody that will conjugate with a specific enzyme, such as peroxidase, alkaline phosphatase, or glucose oxidase. When the enzyme reacts to its specific substrate

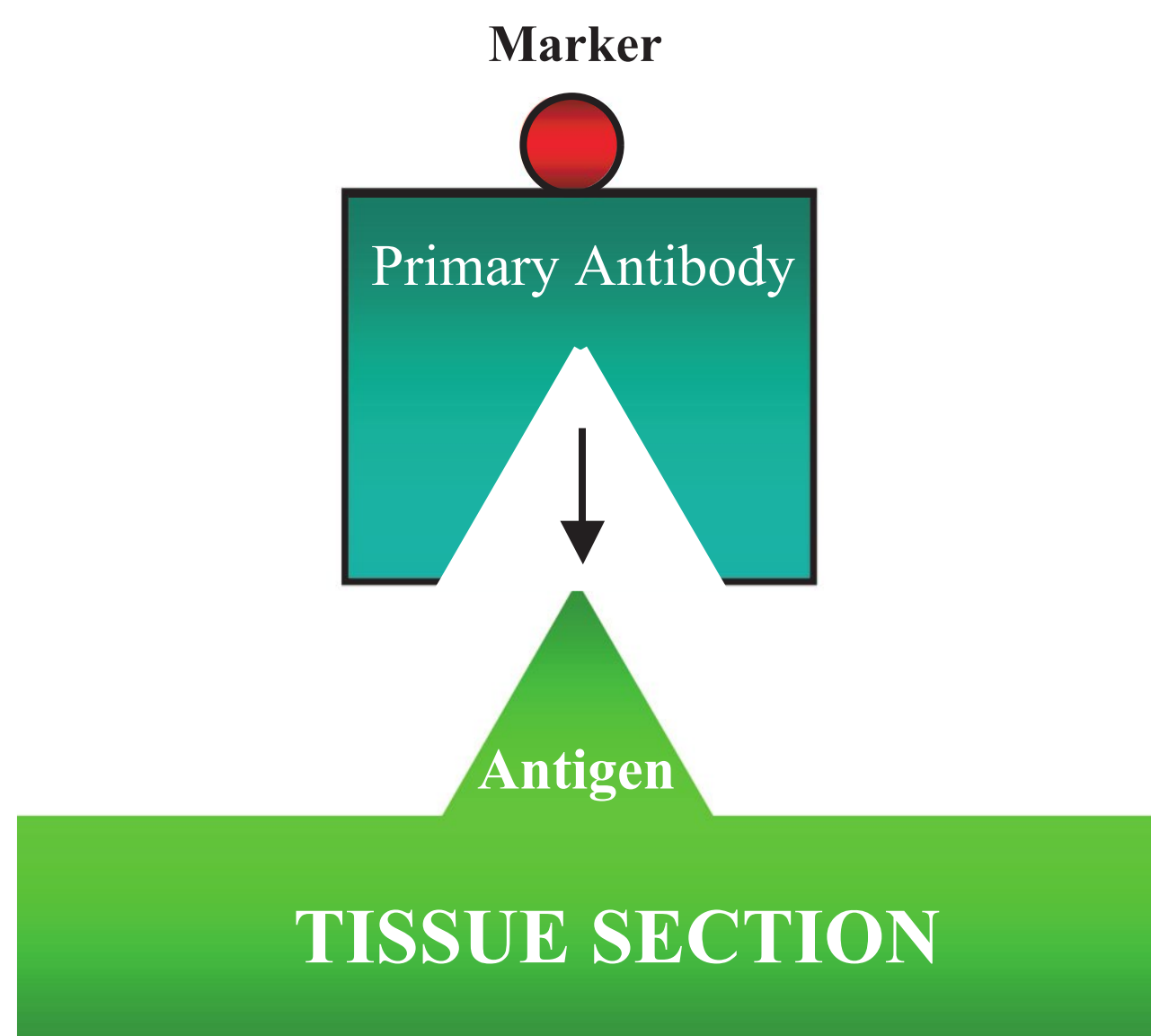


FIGURE 3.1 Direct method is to apply a labeled antibody directly to tissue sections. The marker then demonstrates the cellular location of the antigen in the section.

in the system, a color product will be demonstrated. The indirect method is generally more sensitive than the direct method, mainly because the polyvalent secondary antibody is able to detect multiple sites on the Fc and Fab portions of the primary antibody, and the primary antibody is more accessible to the secondary antibody than

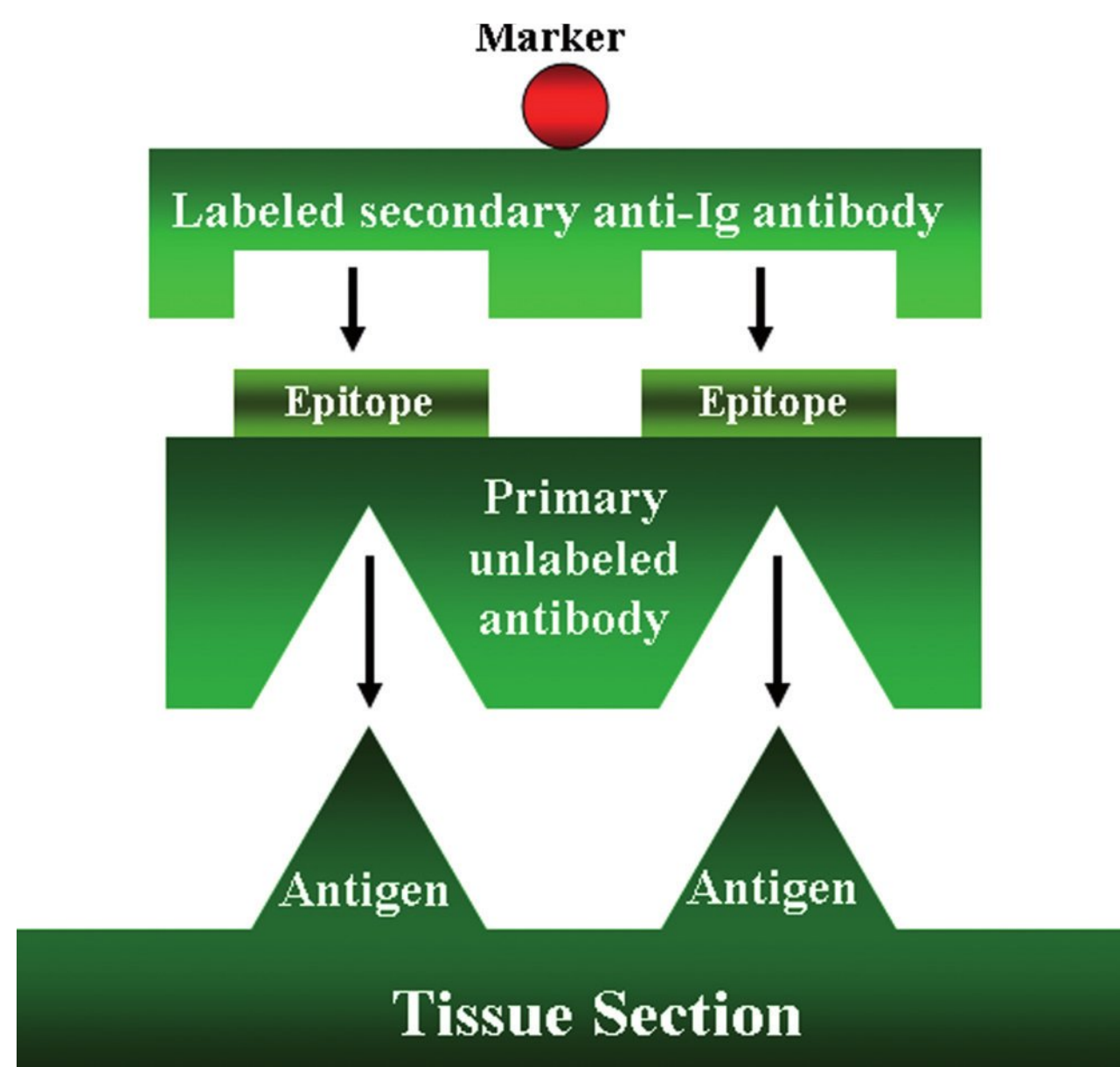


FIGURE 3.2 Indirect labeled method has two layers of antibodies. The primary unlabeled antibody is specific for a particular tissue antigen. The secondary labeled antibody is an anti-immunoglobulin antibody. The marker then identifies the sites of the tissue antigen.

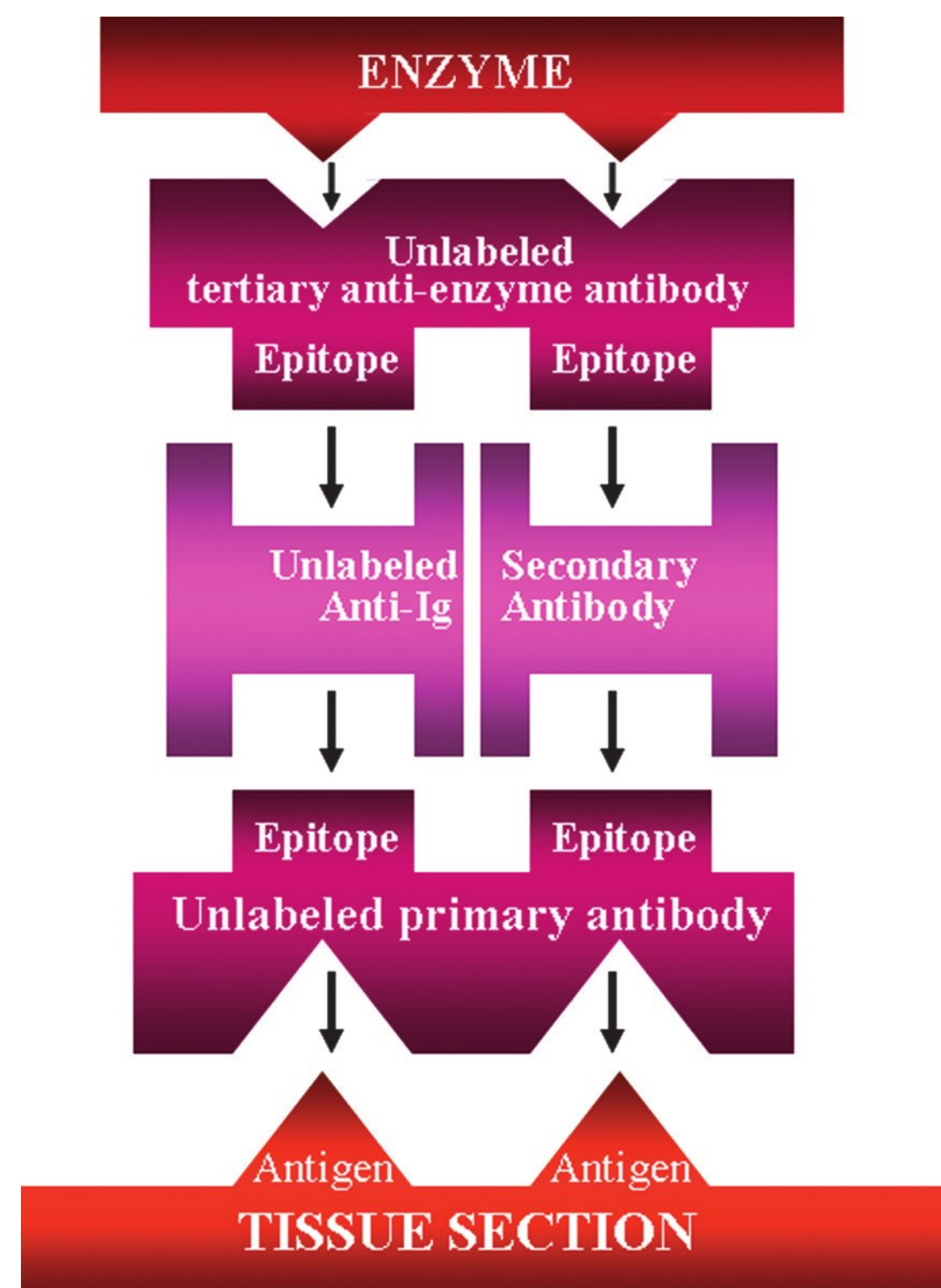


FIGURE 3.3 Unlabeled antibody method has three layers of antibodies. The primary unlabeled antibody is specific for a particular tissue antigen. The secondary unlabeled antibody is an anti-immunoglobulin that reacts to both the primary antibody and the tertiary antibody. The tertiary antibody is an antienzyme antibody, which reacts to a particular enzyme. The enzyme is demonstrated by its reaction with the substrate.

to the tissue antigen, which is being modified by fixation and embedding (16).

Antigen Retrieval and Amplification Techniques
The antigen of interest in tissue sections can be altered after formalin fixation so that it may not react adequately to immunohistochemical staining. The original thinking was that formaldehyde fixation causes an irreversible reduction or even total loss of some antigenic determinants in paraffin sections (19). It is now known that formaldehyde induces alterations in only the tertiary and quaternary structures of protein, but not the primary and secondary structures, so that the epitopes of interest remain intact after formalin fixation (20). The function of the AR technique is, therefore, to restore the tertiary structure of the epitope, making it more accessible to specific antibodies.

The most common AR technique is heating tissue sections in water. The most popular procedure is the microwave heating method (12). Briefly, deparaffinized slides are placed in plastic Coplin jars containing AR solution and are heated in the microwave for 10 minutes. After heating, the slides are washed in phosphate-buffered saline



for 5 minutes and are ready for immunohistochemical staining. The temperature may vary from 90°C to 120°C. The AR solution can be distilled water or various buffered solutions. Besides the microwave, the Coplin jars can be placed in a water bath, pressure cooker, steamer, or autoclave and achieve similar results. The major influencing factors in this technique are the heating temperature, heating time, and pH value of the AR solution.

Proteolytic enzyme digestion also facilitates the increase of immunohistochemical staining sensitivity. For instance, digestion with 0.06% trypsin may restore the glucagon immunoreactivity of formalin-fixed rectal tumors (21), and pretreatment with neuraminidase enhances staining of myelin-associated glycoprotein (22).

The catalyzed reporter deposition (CARD) technique can also be used for signal amplification in immunohistochemistry. The deposition of the biotinylated tyramine (reporter) is through free radical formation, which is catalyzed by oxidizing horseradish peroxidase in the staining system (23). The radicalized biotinylated tyramine will be covalently bound to electron-rich protein residues (tryptophan, histidine, phenylalanine) near the antibody binding site, so that more biotinylated molecules are deposited and the signal is amplified.

Double Immunoenzymatic Techniques

Most of the immunohistochemical stains use only one staining system (e.g., immunoperoxidase). However, in some instances, double staining is desirable. For instance, concurrent demonstration of k- and l-positive cells and estimation of the k/l ratio is useful to determine the clonality of plasma cells. In this circumstance, two immunoenzymatic systems (e.g., immunoperoxidase and immunoalkaline phosphatase) (Fig. 3.4) or one immunoenzymatic system with two different substrates

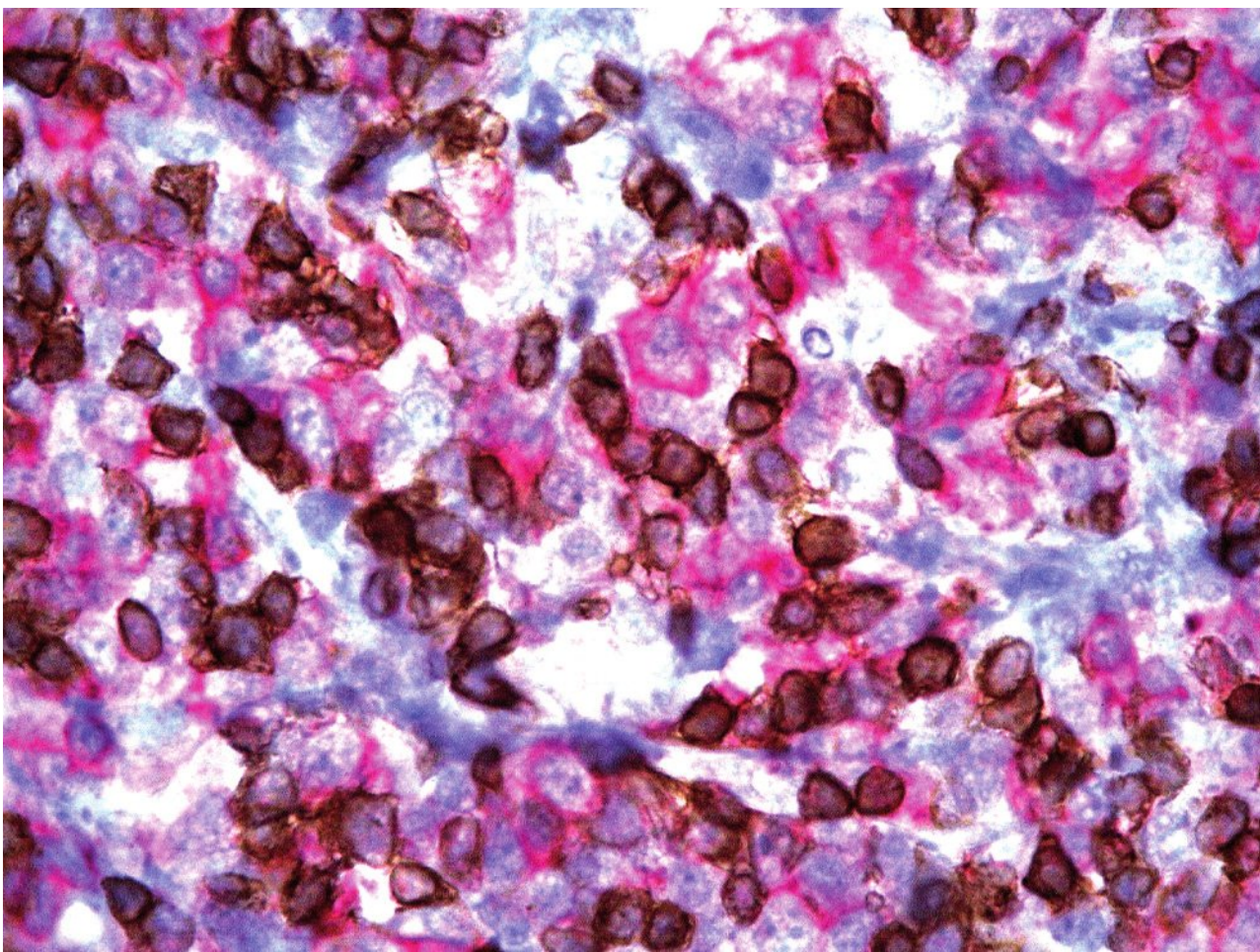


FIGURE 3.4 Lymph node biopsy from a case of T-cell-rich B-cell lymphoma dual-stained for CD3 (brown) and CD20 (red). The large tumor B cells are positive for CD20, whereas the reactive T cells are positive for CD3. Immunoperoxidase-immunoalkaline phosphatase stain, 60× magnification.

TABLE 3.1

The Immunoenzymatic Systems/Immunogold and Their Color Products

Procedure	Color
Peroxidase	
Diaminobenzidine (DAB)	Brown
DAB with enhancement	Black
3-Amino-9-ethyl carbazole (AEC)	Red
4-Chloro-1-naphthol (4-CN)	Blue-black
Hanker–Yates reagent	Blue
a-Naphthol pylonin	Red
3,3',5,5'-Tetramethylbenzidine (TMB)	Blue
Alkaline phosphatase	
Fast blue BB	Blue
Fast red TR	Red
New Fuchsin	Red
BCIP-nitroblue tetrazolium (NBT)	Blue
Glucose oxidase	
Tetrazolium	Blue
Tetranitroblue tetrazolium (TNBT)	Black
Immunogold	
Colloidal gold	Red
With silver enhancement	Black

Modified from Taylor CR, Shi SR, Barr NJ, et al. Techniques of immunohistochemistry: principles, pitfalls and standardization. In: Dabbs D, ed. Diagnostic Immunohistochemistry. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2006:1–42.

(e.g., immunoperoxidase with diaminobenzidine [DAB] and 4-chloro-1-naphthol [4-CN]) can be used. In fact, in the three immunoenzymatic systems, several different substrates are available to produce various chromogens. Therefore, multicolor staining can be potentially achieved. If necessary, immunogold stain can also be added to identify additional antigen in the same sections. The immunoenzymatic systems and immunogold staining and their color products are listed in Table 3.1 (5).

Quality Control

Immunohistochemical staining is a multistep procedure involving tissue procurement, fixation, processing, sectioning, staining, interpreting, and reporting. There are many pitfalls in diagnostic immunohistochemistry as summarized by several review articles (5,24,25). For each step, strict quality control should be observed (5). However, the major responsibilities for the pathologist remain to be correct interpretation and reporting. In this aspect, the positive and negative controls are essential. A positive control is a section that contains the antigen

of interest and is stained the same way as the patient's specimen. A negative control is the same specimen stained with the same procedure as the positive control without including the primary antibody. The primary antibody is usually replaced by the antibody diluent (buffer plus bovine serum albumin carrier protein) or by nonimmune immunoglobulin from the same species of the primary antibody.

False-negative results are obtained when the test tissue is negative but the positive control shows appropriate staining. Under these circumstances, the problem is usually prestaining related, such as improper tissue fixation, processing, pretreatment, or a combination of several factors. When a manual staining procedure is used, the omission of one of the components in the staining may also occur.

False-positive results are obtained when the test tissue and the negative control show positive staining. This is called nonspecific background staining. There are many causes of nonspecific staining, but the most common one is nonspecific ionic binding of antibodies to charged connective tissue elements (5). Sometimes, undissolved precipitates of chromogen or counterstain may also be mistaken as a positive reaction (5).

Selective Use of Monoclonal Antibodies for Staining

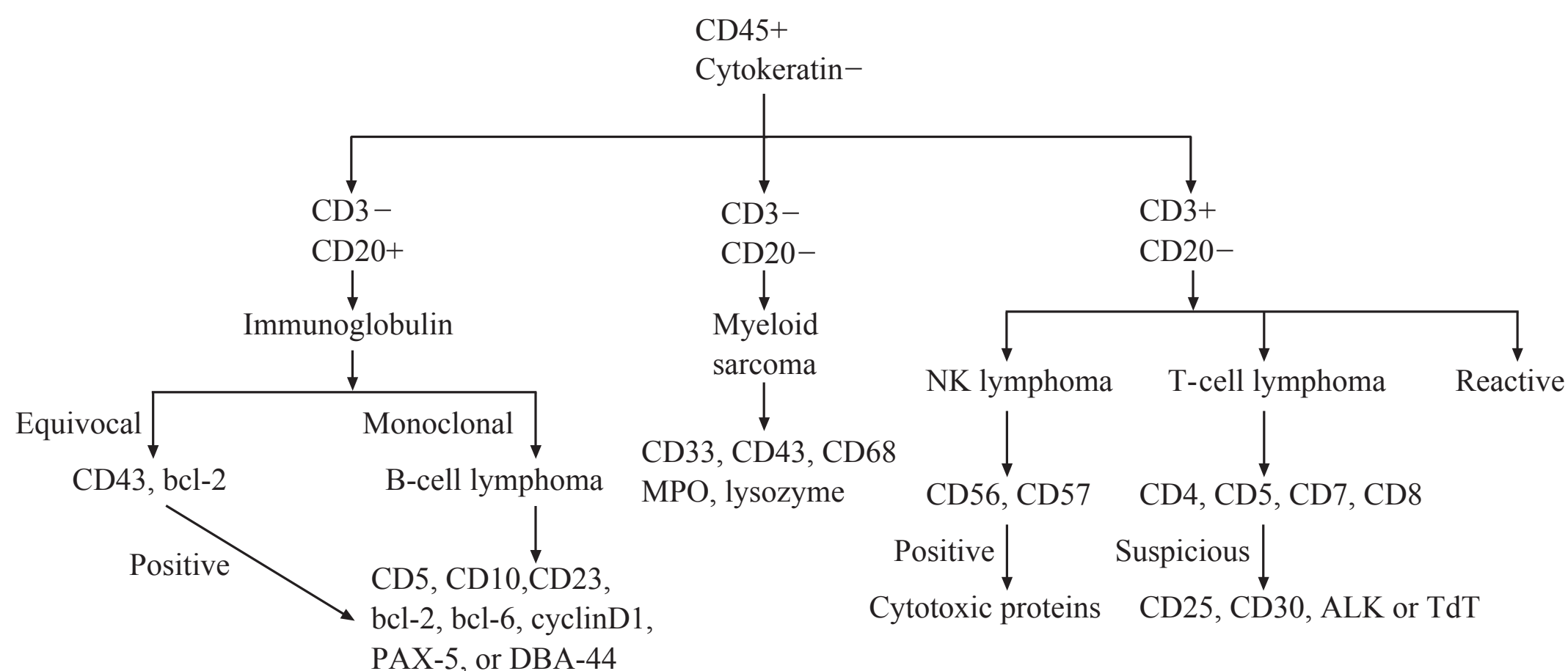
As mentioned before, many antigenic epitopes may become inaccessible after fixation and embedding. Therefore, many of the monoclonal antibodies used for fresh tissue are not applicable for routinely processed tissue sections. Besides using the AR techniques to restore the immunologic reaction, many new antibodies that react to the hidden epitopes are being produced. The most exciting additions are many gene products discovered by recent gene expression studies (26). The increasing numbers of new antibodies for tissue sections greatly enlarge the scope of immunohistochemistry, and some of these antibodies are not yet available for flow cytometry. In addition to the identification of cell lineage, developmental stage and gene products, monoclonal

antibodies can also be used to determine the functional status of tumor cells, such as histone H4 acetylation (26). Automated immunostaining can also be applied to blood and bone marrow smears effectively (27). The currently available monoclonal antibodies are listed in Table 3.2.

Depending on the experience of the pathologist and the difficulty of the diagnosis, variable numbers of antibodies can be selected in individual cases (25,28,29); thus, large screening or standard panels for all cell lineages are not necessary and wasteful. There are basically two approaches for antibody selection (24). The first approach is the algorithmic approach by multistep screening. The decision in every step depends on the results of the antibody reactions in the preceding step. The general practice is to work on several steps at the same time and to interpret the results according to the algorithmic sequence. The second approach is a panel approach on the basis of a preliminary diagnosis either clinically or morphologically. The antibody panel is construed not only for the targeted diagnosis but also for the possible differential diagnoses. The second approach is more suitable for experienced pathologists.

The following are some suggestions for the algorithmic approach (Algorithm 3.1). In a poorly differentiated tumor, CD45 (LCA) and pancytokeratin are the proper antibodies for screening. Occasionally, S100 should be included to rule out nonmelanotic melanoma. If pancytokeratin is positive, the pathologist will select antibodies to distinguish various types of epithelial tumors. When CD45 is positive, the tumor is considered to be possibly a hematologic neoplasm, and CD3 (Fig. 3.5) and CD20 (Fig. 3.6) should be ordered to identify the T-cell and B-cell lineage. The lymphoid cells being predominantly positive for CD3 usually represent reactive T lymphocytes, unless those positive cells are morphologically neoplastic.

However, most of the lymphomas are of B-cell origin; therefore, if CD20 staining is not confined to the follicular area of the lymph node or the mucosa-associated lymphoid tissue, lymphoma should be considered. Immunoglobulin staining will identify the clonality of the B cells, and the presence of a monoclonal B-cell population in most



ALGORITHM 3.1 Algorithmic approach for immunodiagnosis. MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.



TABLE 3.2

Monoclonal Antibodies Used in Immunohistochemistry

CD/ antigen	Cell specificity	Clinical application
ALK	ALCL cell	ALCL
Annexin A1	Hairy cell	Hairy cell leukemia
b-F1	T-cell receptor	T-cell lymphoma/leukemia
Bcl-2	B cell	B lymphoma
Bcl-6	B cell	B lymphoma
CD1a	Thymocyte, Langerhans cell	Precursor T-cell lymphoma/leukemia
CD3	T cell	T lymphoma/leukemia
CD4	T helper cell	T lymphoma/leukemia
CD5	T cell	T lymphoma/leukemia
CD8	T suppressor cell	T lymphoma/leukemia
CD10	Immature B cell	ALL, lymphoma of follicular center cell origin
CD15	Reed–Sternberg and myeloid cells	Hodgkin lymphoma
CD20	B cell	B lymphoma
CD21	FDC	FDC tumor and follicle identification
CD23	B cell, FDC	B lymphoma
CD30	Reed–Sternberg and activated T/B cells	Hodgkin lymphoma
CD34	Hematopoietic stem cell	Acute lymphoid/myeloid leukemia
CD35	FDC	FDC tumor and follicle identification
CD38	Plasma cell, activated T & B cells	Myeloma
CD42b	Platelet/megakaryocyte	Acute megakaryoblastic leukemia
CD43	T cell, B cell subset	T/B-cell lymphoma, myeloid sarcoma
CD45	All leukocytes	Lymphomas, leukemias
CD45RA	T cell, B cell subset	T/B-cell lymphoma
CD45RO	T cell, B cell subset	T/B-cell lymphoma
CD56	NK cell	NK/T-cell lymphoma/leukemia
CD57	NK cell	NK/T-cell lymphoma/leukemia
CD61	Platelet/megakaryocyte	Acute megakaryoblastic leukemia
CD68	Monocyte/histiocyte	Monocyte/histiocyte tumors
CD79a	B cell	B lymphoma
CD79b	B cell	B lymphoma
CD117	Hematopoietic stem cell, mast cell	Acute myeloid leukemia, mast cell disease
CD123	Hairy cell, plasmacytoid dendritic cell, basophil	Hairy cell leukemia, blastic plasmacytoid dendritic cell neoplasm, acute basophilic leukemia
CD138	Plasma cell, B cell	Myeloma, lymphoma with plasma cells
CD163	Monocyte	Monocytic or myelomonocytic leukemias
Cyclin D1	B cell	Mantle cell lymphoma
DBA-44	B cell	Hairy cell leukemia
EMA	Epithelial cell	Carcinoma, ALCL
Gr-B	Cytotoxic T cell, NK cell	T-cell lymphoma/leukemia, NK cell tumors
Kappa	Plasma cell, B cell	Myeloma, B-cell lymphomas

(continued)

TABLE 3.2 (continued)

Monoclonal Antibodies Used in Immunohistochemistry

CD/ antigen	Cell specificity	Clinical application
Ki-67	Proliferation fraction	High-grade lymphomas
Lambda	Plasma cell, B cell	Myeloma, B-cell lymphomas
MUM1	Plasma cell, B and T cells	Myeloma, some B- and T-cell lymphomas
PAX5/BSAP	B cell	Hodgkin and non-Hodgkin lymphomas
TdT	Precursor T/B cells	Precursor T/B-cell lymphoma/leukemia
TIA-1	Cytotoxic T cell, NK cell	T-cell lymphoma/leukemia, NK cell tumors
TRAcP	Lymphoid cells	Hairy cell leukemia

ALK, anaplastic lymphoma kinase; ALCL, anaplastic large cell lymphoma; EMA, epithelial membrane antigen; FDC, follicular dendritic cell; NK, natural killer; Gr-B, granzyme-B; TdT, terminal deoxynucleotidyl transferase; TIA-1, T-cell intracellular antigen; TRAcP, tartrate-resistant acid phosphatase.

occasions represents a B-cell lymphoma. Unfortunately, monoclonal surface immunoglobulin pattern is mainly demonstrated in plasma cell tumors or B cells with abundant cytoplasm, such as immunoblasts. Many lymphomas do not show a definitive immunoglobulin staining. In those cases, bcl-2 and CD43 staining can be helpful. CD43 is a T-cell marker, but its coexpression with CD20 is highly suggestive of a B-cell lymphoma (except in the Peyer patch) (Fig. 3.7) (28,30). Bcl-2 is specific for follicular lymphoma, but its expression in a B-cell population is also seen in other B-cell tumors. When a B-cell lymphoma is suspected, additional specific markers such as CD10, CD5, CD23, bcl-6, cyclin D1, PAX5, and DBA-44 can be used for subclassification (31).

For T-cell tumors, CD4, CD5, CD7, CD8, CD25, CD30, terminal deoxynucleotidyl transferase (TdT), and

anaplastic lymphoma kinase (ALK) can be used selectively, depending on the morphology. If natural killer (NK)/T-cell lymphoma is in the differential diagnosis, CD56 and CD57 should be included in the immunohistochemical panel.

An immunophenotype of CD45⁺, CD3[−], CD20[−] is highly suggestive of myeloid sarcoma, and CD33, MPO, lysozyme, CD68, and CD43 should be ordered. Occasionally, this immunophenotype can be demonstrated in plasmacytoma, but this tumor usually shows weak or negative CD45 staining. For suspected AML in the bone marrow, CD34 and CD117 may help to identify the blasts, but these markers can stain for immature cells other than myeloblasts. Lymphoblasts and hematogones are positive for CD34. Immature normoblasts are positive for CD117 (32). Some cases of myeloma are also positive for CD117. On the other hand, the number of blasts can be

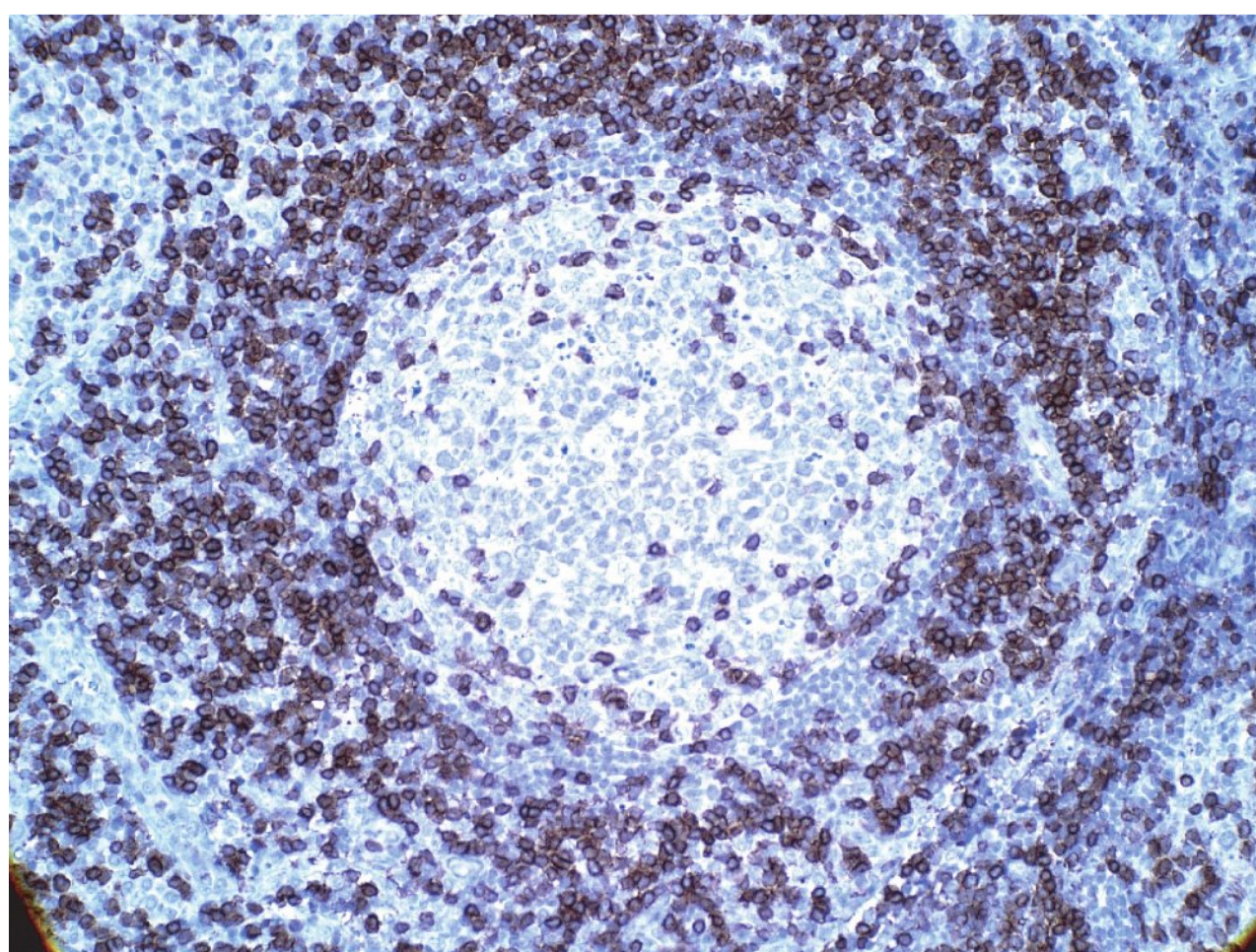


FIGURE 3.5 Normal lymph node biopsy stained for CD3, showing interfollicular staining with partial involvement of the mantle zone. The germinal center is spared. Immunoperoxidase, 20× magnification.

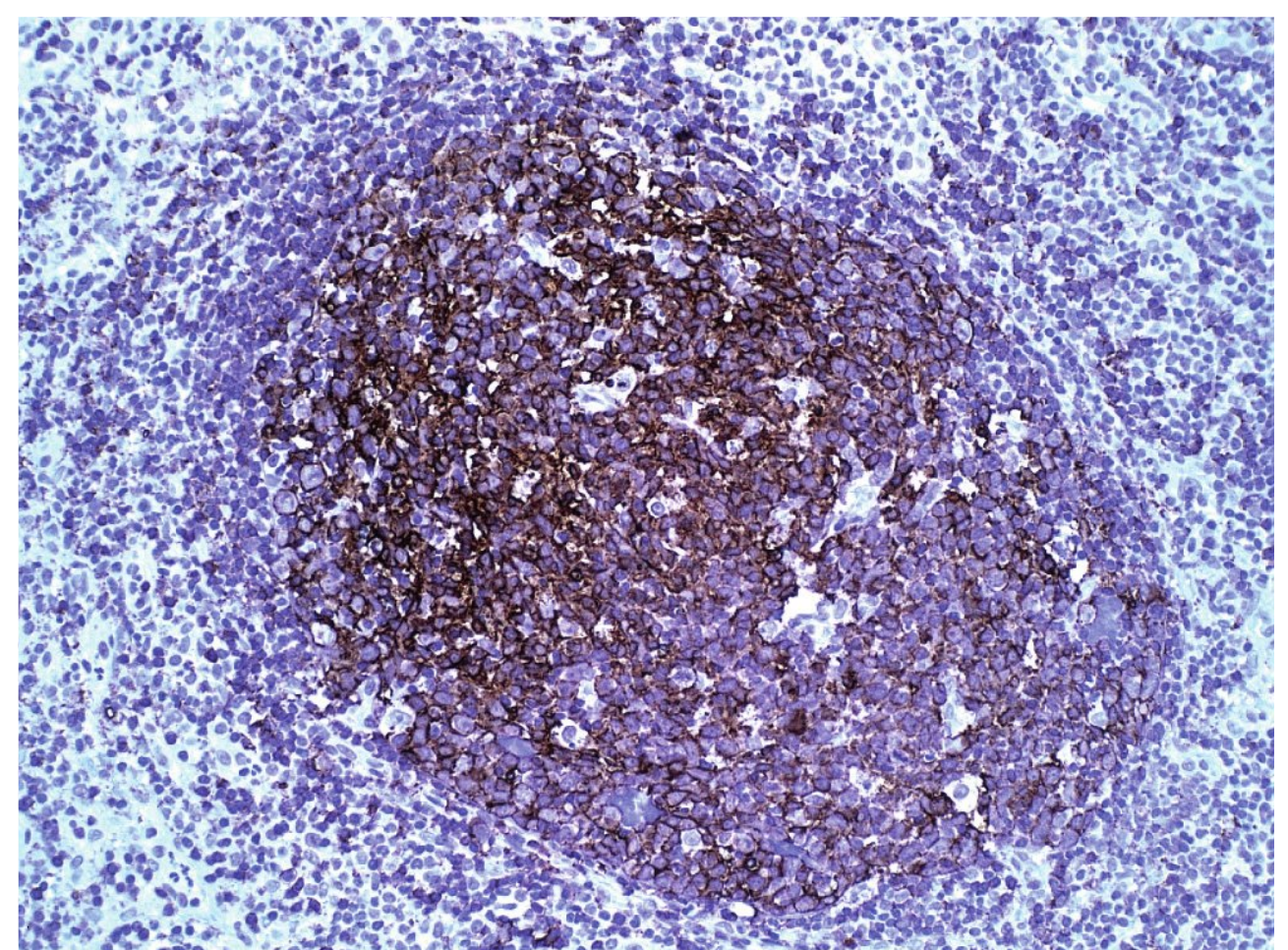


FIGURE 3.6 Normal lymph node biopsy stained for CD20, showing strong staining of the germinal center cells and partial involvement of the mantle zone. Immunoperoxidase, 20× magnification.

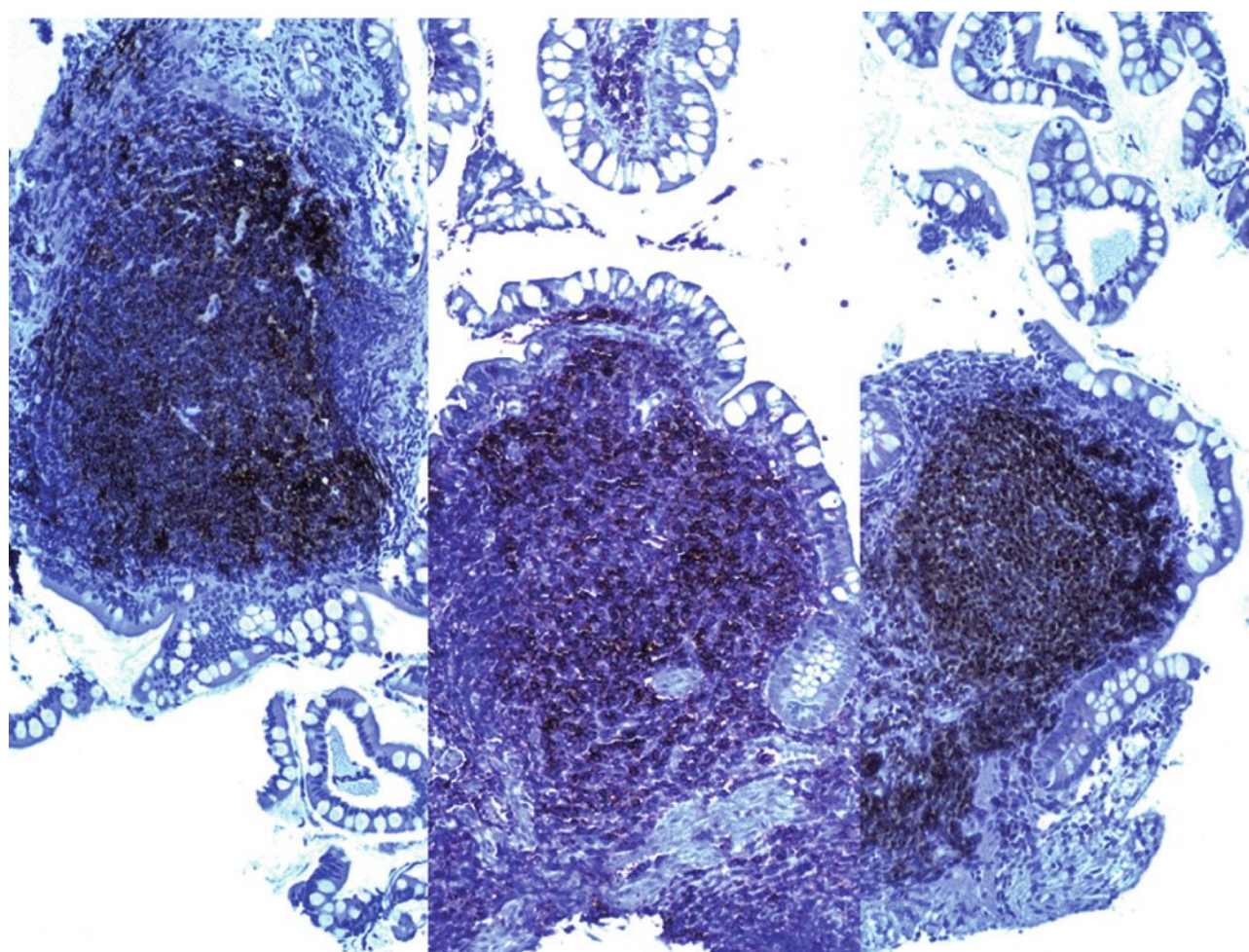


FIGURE 3.7 Biopsy of terminal ileum showing a Peyer patch, which reacts to CD20 (**left**), bcl-2 (**center**), and CD43 (**right**). Immunoperoxidase, 10× magnification.

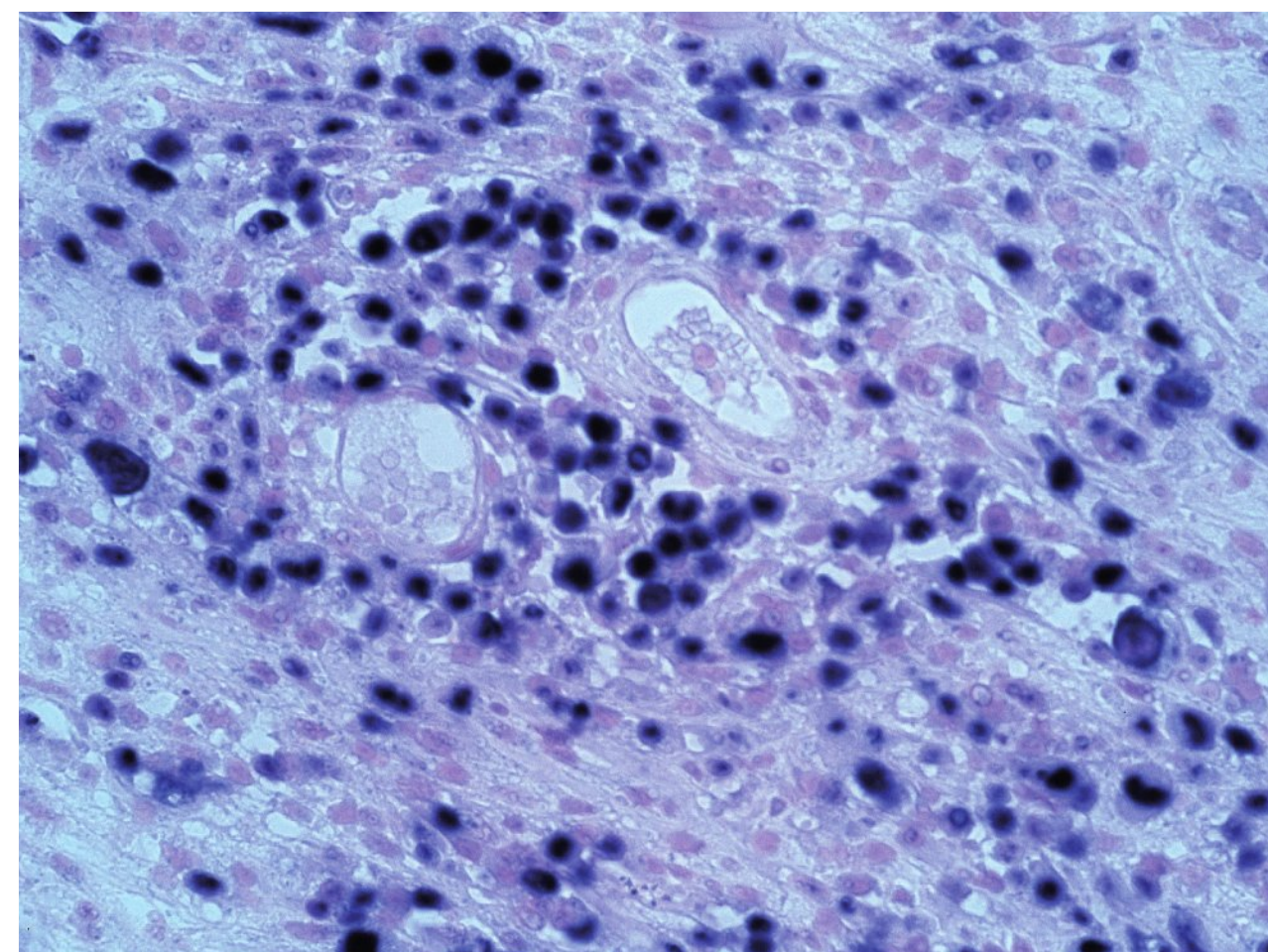


FIGURE 3.8 Brain biopsy of a case of posttransplant lymphoproliferative disorder showing Epstein-Barr virus staining. RNA in situ hybridization, 40× magnification.

underestimated with immunohistochemistry. There are several comprehensive review articles for application of immunohistochemistry in the diagnosis of hematologic neoplasms. The review by Garcia and Swerdlow (31) discusses the general principle of immunohistochemistry in the diagnosis of lymphoid tumors, the review by Kremer et al. (32) is confined to its use in bone marrow specimens, and the article by O'Malley et al. (33) devotes to its application to spleen specimens.

IN SITU HYBRIDIZATION

In situ hybridization is frequently incorporated with immunofluorescence techniques for the demonstration of chromosomal abnormalities, which is called fluorescence in situ hybridization (FISH) and is the most popular technique for cytogenetic studies. Isotopes can also be used for probe labeling to demonstrate different kinds of nucleic acids. In histologic examination, the most useful technique is NISH (16,34). This technique is mainly used to identify DNA and/or RNA of microorganisms, especially viruses. In hematopathology, it is helpful to demonstrate Epstein-Barr virus in various types of lymphomas (Fig. 3.8). The recent application of NISH to identify immunoglobulin light-chain messenger RNA is particularly useful in cases where regular immunoglobulin staining is weak or equivocal (Fig. 3.9).

The first step in nucleic acid hybridization is to break the double-stranded DNA into two single strands with high temperature, which is called denaturation. A DNA probe is then added to the preparation, and if it is complementary to the targeted DNA, the probe will hybridize with single-stranded DNA from the patient specimen to reform a double-stranded DNA. This hybridized product is then demonstrated by the probe label,

such as biotin or enzymes. This procedure is applicable for both DNA and RNA. Because RNA cannot be cloned, the enzyme reverse transcriptase has to be used to convert RNA to a complementary copy of DNA (cDNA) to make probes for the detection of RNA viruses or immunoglobulin RNA.

There are several strategies for the improvement of in situ hybridization techniques. These strategies include target amplification (in situ polymerase chain reaction, primed labeling, self-sustained sequence replication), signal amplification (tyramide signal amplification, branched DNA amplification), and probe amplification (padlock probes and rolling circle amplification). The potential of this technology is highly promising.

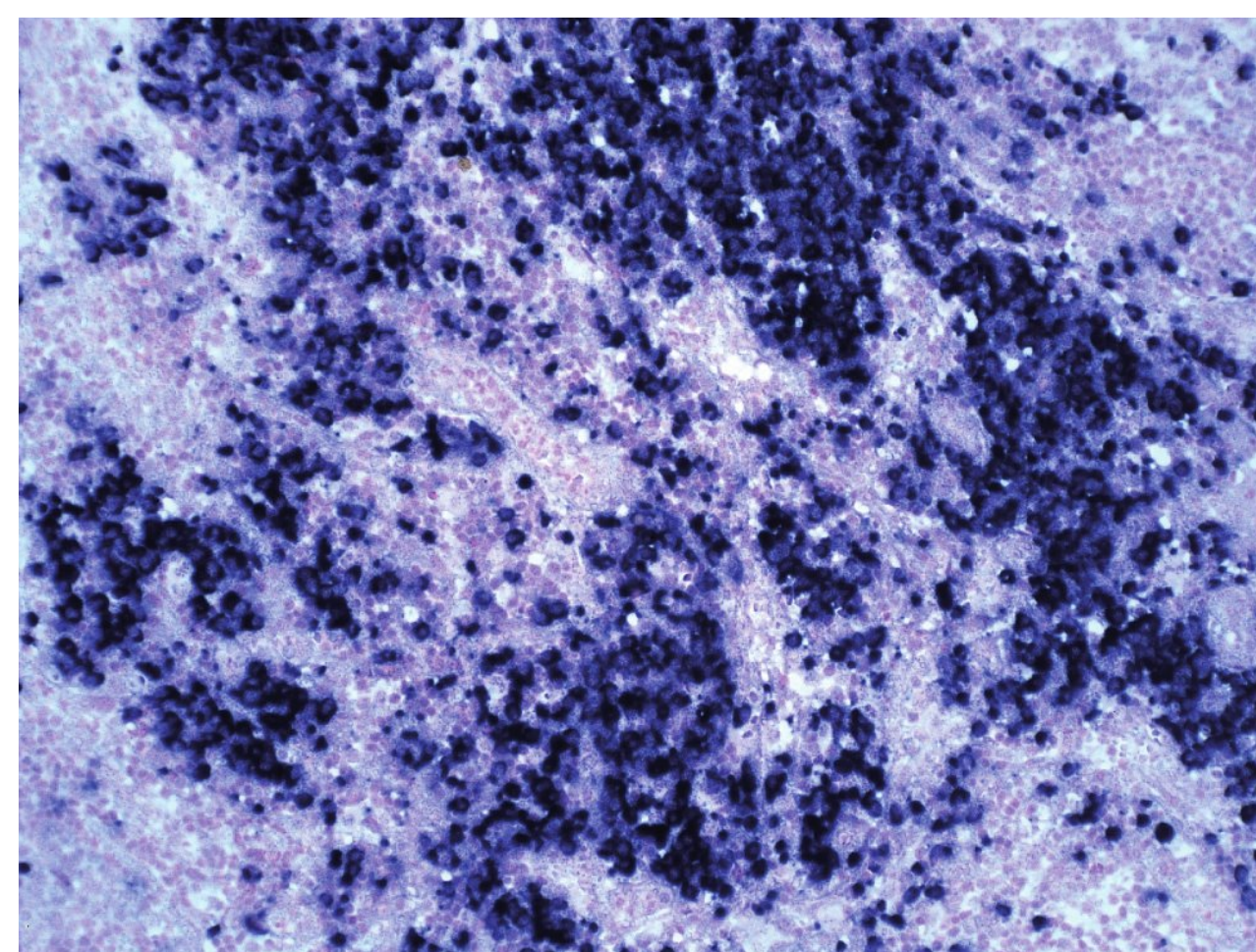


FIGURE 3.9 Lymph node biopsy of a case of lymphoplasmacytic lymphoma, showing diffuse strong kappa light-chain staining. RNA in situ hybridization, 20× magnification.

REFERENCES

- Bennett JM, Catovsky D, Daniel MT, et al. French-American-British (FAB) Cooperative Group. Proposals for the classification of the acute leukemias. *Br J Haematol*. 1976;33:451–458.
- Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia. *Ann Intern Med*. 1985;103:626–629.
- Li CY, Yam LT, Sun T. Modern Modalities for the Diagnosis of Hematologic Neoplasms. New York, NY: Igaku-Shoin; 1996:7–26.
- Elghetany MT, MacCallum JM, Davey FR. The use of cytochemical procedures in the diagnosis and management of acute and chronic myeloid leukemia. *Clin Lab Med*. 1990;10:707–720.
- Taylor CR, Shi SR, Barr NJ, et al. Techniques of immunohistochemistry: principles, pitfalls and standardization. In: Dabbs D, ed. *Diagnostic Immunohistochemistry*. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2006:1–42.
- Coon AH, Creech HJ, Jones RN. Immunological properties of an antibody containing a fluorescent group. *Proc Soc Exp Biol Med*. 1941;47:200–202.
- Avrameas S. Enzyme markers: their linkage with proteins and use in immunohistochemistry. *Histochem J*. 1972;4:321–330.
- Taylor CR, Burns J. The demonstration of plasma cells and other immunoglobulin containing cells in formalin-fixed, paraffin-embedded tissues using peroxidase labeled antibody. *J Clin Pathol*. 1974;27:14–20.
- Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature*. 1975;256:495–497.
- Huang SN. Immunohistochemical demonstration of hepatitis B core and surface antigens in paraffin sections. *Lab Invest*. 1975;33:88–95.
- Leong ASY. Applications of microwave irradiation in histopathology. *Pathol Ann*. 1988;2:213–234.
- Shi SR, Key ME, Kalra KL. Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. *J Histochem Cytochem*. 1991;39:741–748.
- Walker RA. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment I. *Histopathology*. 2006;49:406–410.
- Taylor CR, Levenson RM. Quantification of immunohistochemistry—issues concerning methods, utility and semiquantitative assessment II. *Histopathology*. 2006;49:411–424.
- Dabbs D, ed. *Diagnostic Immunohistochemistry. Therapeutic and Genomic Applications*. 3rd ed. Philadelphia, PA: Saunders; 2010.
- Elias JM. *Immunohistopathology: A Practical Approach to Diagnosis*. 2nd ed. Chicago, IL: ASCP Press; 2003.
- Williams JH, Mepham BL, Wright DH. Tissue preparation for immunocytochemistry. *J Clin Pathol*. 1997;50:422–428.
- Friesen SR, Kimmel JR, Tomita T. Pancreatic polypeptide as a screening marker for pancreatic peptide apudomas in multiple endocrinopathies. *Am J Surg*. 1980;139:61–72.
- Leong ASY, Gilham PN. The effects of progressive formaldehyde fixation on the preservation of tissue antigens. *Pathology*. 1989;21:266–268.
- Dill KA, Shortle D. Denatured state of proteins. *Annu Rev Biochem*. 1991;60:795–825.
- Helander KG. Kinetic studies of formaldehyde binding in tissue. *Biotech Histochem*. 1994;69:177–179.
- Tanaka M, Sato S, Baba H, et al. Effect of neuraminidase on reactivity of anti-myelin-associated glycoprotein (MAG) antiserum with human natural killer cells. *Biomed Res*. 1984;5:283–285.
- Toda Y, Kono K, Abiru H, et al. Application of tyramide signal amplification system to immunohistochemistry: a potent method to localize antigens that are not detectable by ordinary method. *Pathol Int*. 1999;49:479–483.
- Leong SYA. Pitfalls in diagnostic immunohistology. *Adv Anat Pathol*. 2004;11:86–93.
- Yaziji H, Barry T. Diagnostic immunohistochemistry: what can go wrong? *Adv Anat Pathol*. 2006;13:238–246.
- Advani AS, Gibson SE, Douglas E, et al. Histone H4 acetylation by immunohistochemistry and prognosis in newly diagnosed adult acute lymphoblastic leukemia (ALL) patients. *BMC Cancer*. 2010;10:387–394.
- Happerfield LC, Saward R, Grimwade L, et al. Automated immunostaining of cell smears: an alternative to flow cytometry. *J Clin Pathol*. 2008;61:740–743.
- Gudgin EJ, Erber WN. Immunophenotyping of lymphoproliferative disorders: state of the art. *Pathology*. 2005;37:457–478.
- Gocke CD. Immunohistology of non-Hodgkin lymphoma. In: Dabbs D, ed. *Diagnostic Immunohistochemistry*. 2nd ed. Philadelphia, PA: Churchill Livingstone; 2006:137–161.
- Beneck LPS, Weisberger J, Gorczyca W. Coexpression of CD43 by benign B cells in the terminal ileum. *Appl Immunohistochem Mol Morphol*. 2005;13:138–141.
- Garcia CF, Swerdlow SH. Best practices in contemporary diagnostic immunohistochemistry. Panel Approach to hematolymphoid proliferations. *Arch Pathol Lab Med*. 2009;133:756–765.
- Kremer M, Quintanilla-Martinez L, Nahrig J, et al. Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis. *Virchow Arch*. 2005;447:920–937.
- O'Malley DP, Kim YS, Perkins SL, et al. Morphologic and immunohistochemical evaluation of splenic hematopoietic proliferations in neoplastic and benign disorders. *Mod Pathol*. 2005;18:1550–1561.
- Qian X, Lloyd RV. Recent developments in signal amplification methods for in situ hybridization. *Diagn Mol Pathol*. 2003;12:1–13.

Molecular Genetics

4

In recent years, molecular genetics has played an increasingly important role in the diagnosis and prognostication of lymphomas and leukemias (1–5). The role of molecular genetics, however, may differ from one entity to another and can be divided into three categories:

1. Mandatory for diagnosis: For the diagnosis of Burkitt lymphoma and chronic myelogenous leukemia (CML), MYC oncogene and BCR-ABL1, respectively, are the absolute diagnostic markers. There are no substitutes for these markers that are acceptable for a definitive diagnosis. Many subtypes of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are also defined by cytogenetic karyotypes (6). AML with t(8;21)(q22;q22), inv(16)(p13;q22) or t(16;16)(p13;q22), and t(15;17)(q22;q12) are so characteristic that they supersede the blast count for the diagnosis of AML. These karyotypes also confer a good prognosis.
2. Markers for treatment or prognosis: For the diagnosis of acute promyelocytic leukemia (AML-M3) a morphologic diagnosis is possible, but the identification of t(15;17)(q22;q12) or promyelocytic leukemia/retinoic acid receptor alpha (PML-RARα) is not only important for the confirmation of the diagnosis but also for the treatment. If the leukemic cells carry other variants, such as t(11;17), patients will not respond to all-trans-retinoic acid treatment (6). For anaplastic large-cell lymphoma, the anaplastic lymphoma kinase (ALK) gene is not necessarily present in each case, but the presence or absence of this gene will greatly influence the clinical course, the therapeutic strategy, and the prognosis (7).
3. Confirmation of diagnosis: For mantle cell lymphoma (MCL), there are many morphologic and immunologic criteria for the diagnosis, but the results of these tests are frequently equivocal, and the demonstration of t(11;14)(q13;q32) or B-cell lymphoma/leukemia-1/immunoglobulin H (BCL-1/IgH) is essential for a definitive diagnosis (8). Lymphoplasmacytic lymphoma is usually diagnosed by exclusion of many other lymphomas, but the detection of t(9;14)(p13;q32) or PAX5/IgH will confirm the diagnosis in cases of nonsecretory lymphoplasmacytic lymphoma (cases without Waldenström macroglobulinemia) (9).

CYTOGENETICS

Cytogenetics plays multiple roles in relation to hematologic neoplasms (4,10,11). In clinical practice, the most useful occasion for cytogenetics is in determining the nature of a tumor in equivocal cases when immunophenotyping or immunogenotyping demonstrated monoclonality of the tumor and yet morphologic evidence of malignancy is lacking. In these cases, an abnormal karyotype casts a decisive vote for malignancy (10,12). Even when monoclonality is not identified, chromosomal aberration is a strong indication of malignancy or premalignancy (10,11).

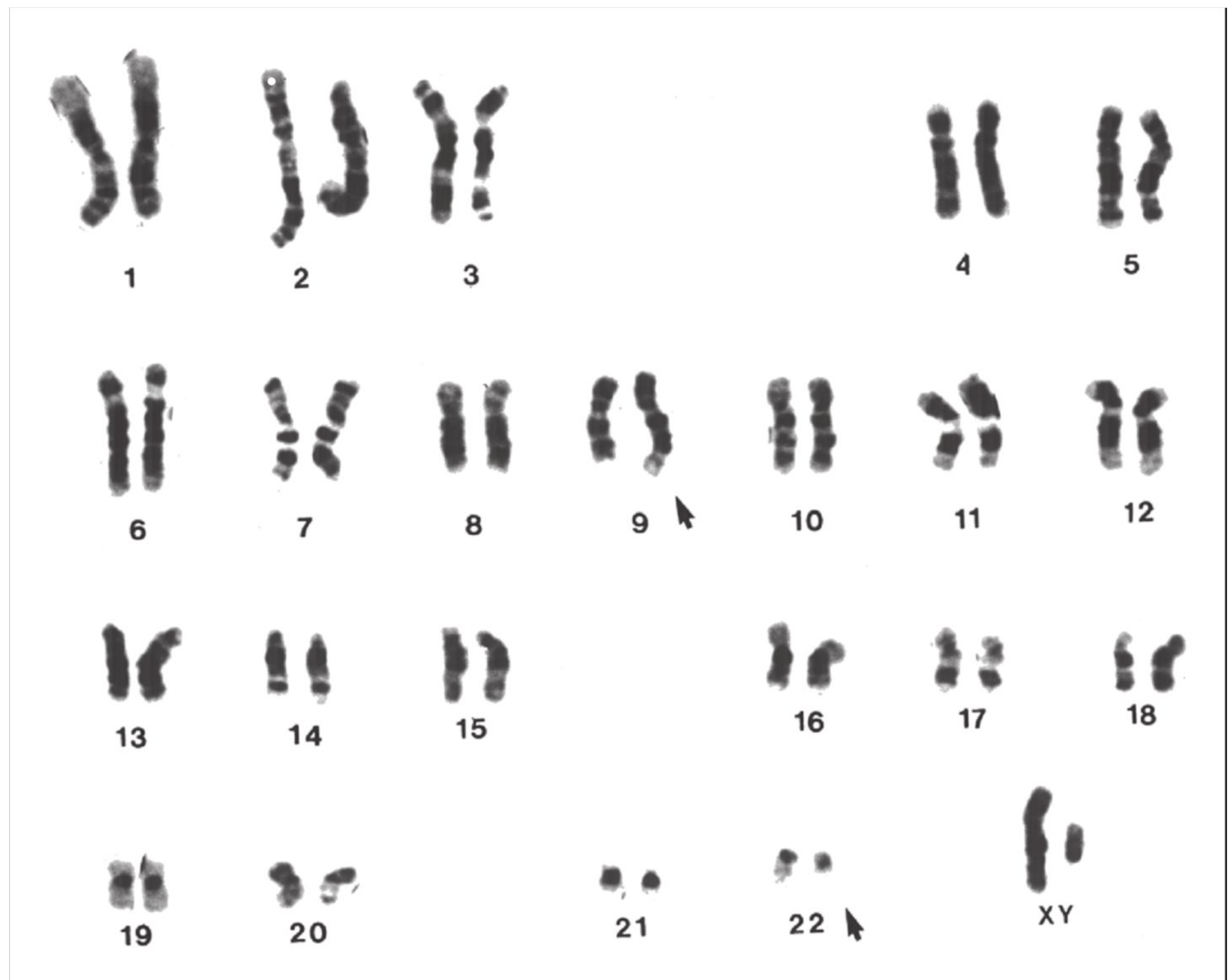
Although most lymphomas and leukemias still have no diagnostic chromosomal pattern, karyotyping is the most reliable tool for a definitive diagnosis of many hematologic neoplasms. As mentioned before, it is particularly useful for the diagnosis of CML, Burkitt lymphoma, AML, MCL, and anaplastic large cell lymphoma, just to name a few (Fig. 4.1).

Chromosomal aberrations are classified into numeric and structural abnormalities. Structural abnormalities include translocations, deletions, inversions, duplications, and isochromosomes. Among these, reciprocal translocation is most commonly found in hematologic neoplasms. This translocation involves the exchange of segments between two chromosomes; the total number of chromosomes is unchanged.

The numeric abnormalities are subdivided into polyploid and aneuploid. The term polyploid refers to multiplication of the normal haploid number of 23. Thus diploidy refers to 46 chromosomes, triploidy 69, and tetraploidy 92. Aneuploid, in contrast, refers to multiplication of chromosomes by irregular numbers, for instance, monosomy and trisomy. As the result of monosomy in a single chromosome, the total number of chromosome becomes 45. When trisomy is present in a single chromosome, the number of chromosome becomes 47.

In addition, cytogenetics is a reliable predictor for prognosis. Generally speaking, neoplasms carrying a normal karyotype have a better prognosis than those with an abnormal one. There are, however, a few exceptions.

FIGURE 4.1 Karyotype of a case of CML, showing t(9;22) translocation (arrow), (Philadelphia chromosome). (Courtesy of Dr. P. Koduru, North Shore University Hospital, New York.)



For instance, cases of anaplastic large-cell lymphoma that express t(2;5) have a more favorable prognosis than those without this karyotype (7). In childhood ALL, cases with hyperdiploidy carry a better prognosis than do diploidy cases (13). The prediction of prognosis by karyotyping is sometimes associated with the histologic pattern. For instance, a favorable prognosis with t(14;18) is due to its association with follicular lymphoma, whereas the poor prognosis predicted by t(8;14) is due to its association with Burkitt lymphoma (14). Some numeric chromosomal abnormalities, such as +5, +6, or +8, are related to shorter survival in patients with non-Hodgkin lymphoma (15). In AMLs and myelodysplastic syndromes, karyotyping is routinely performed even in entities without a specific karyotype, because the presence or absence of chromosomal aberration and the presence of a particular karyotype are important to stratify the risk groups and guide the treatment.

Cytogenetic studies are also useful in monitoring clinical course. When the patient has a relapse, karyotyping is able to determine whether the tumor is recurrent or secondary (16). Structural chromosomal abnormalities usually initiate malignant transformation, whereas numeric chromosomal abnormalities represent chromosomal evolution leading to the progression of disease (17). The appearance of new abnormalities in the karyotype, no matter whether the abnormality is structural or numerical, signals transformation of the tumor to a higher grade malignancy (10).

Finally, cytogenetic analysis is also able to detect residual minimal disease. The detection of residual tumor cells is frequently based on the use of molecular genetic

techniques, such as polymerase chain reaction (PCR) or the fluorescence in situ hybridization (FISH) technique.

Fluorescence in Situ Hybridization

Conventional karyotyping requires fresh specimens, which are cultured to harvest cells in the metaphase for chromosome analysis. Therefore, it is time consuming and costly, and it frequently obtains unsatisfactory results because of the failure in cell growth or because the normal population overgrows the tumor cells. FISH overcomes all of these deficiencies of karyotyping (11,18) and can identify the cytogenetic abnormality in cells at the interphase. Therefore, it can be applied to dried smears of bone marrow, peripheral blood, body fluids, and paraffin sections. Because cell culture is not required, the failure rate is very low. However, FISH can also be applied to cultured cells in metaphase under special conditions. A large number of cells can be analyzed by FISH so that a percentage of positive cells can be calculated. Therefore, this analysis is particularly useful in therapeutic monitoring. FISH is more sensitive than karyotyping as it can detect cryptic or masked translocations. For instance, 5% of patients with CML have a Philadelphia chromosome from a submicroscopic insertion translocation. Therefore, the aberration is not detectable by conventional karyotyping but is readily detected by FISH (11).

FISH involves the binding of fluorochrome-labeled DNA probes to target DNA or RNA sequences inside fixed cells. The basic principle is that a single-stranded DNA can bind to a complementary single-stranded DNA, and this hybridization process can be demonstrated with fluorochrome labels. Normal cells contain two alleles of

each chromosome; therefore, two signals for a certain chromosome can be demonstrated by a specific DNA probe in normal cells. When one signal or three signals representing the same chromosome are demonstrated in individual cells, this represents numeric abnormalities (monosomy or trisomy) (Figs. 4.2 and 4.3). Chromosomal translocation can be demonstrated by showing two overlapped signals from two different genes (Fig. 4.4). Chromosomal inversion can be detected in the culture cells at the metaphase.

Depending on the targeted abnormalities, a certain probe should be selected (11). For the detection of numeric abnormalities, the centromere-specific probes should be used. For the detection of structural abnormalities, such as reciprocal translocations and inversions, locus-specific probes are most frequently used. Telomere-specific probes are used to detect subtle chromosome abnormalities that involve the ends of chromosomes. Whole chromosome-specific paints with sequence DNA probes are used to identify marker chromosomes and to detect cryptic translocations.

FISH probes can be used with different strategies, including single fusion, double fusion, break-apart, and extra-signal strategy. The break-apart FISH strategy uses different colored probes that hybridize to sequences flanking both sides of the common breakpoint region. In normal nuclei, this signal pattern would appear as two fusion signals. Any structural abnormality that alters the breakpoint region separates these signals, changing both the number and color of the signals in the nucleus. In those cases, the fusion signals are replaced by red or orange signals. This strategy is particularly useful to detect translocation of a gene with many potential partner genes. For technical details, the reader is referred to (11).

Array-Based Whole Genome Scanning Technologies

Array-based technologies can be used for karyotyping and are complementary to conventional karyotyping. These tools do not rely on cell division, have super resolution

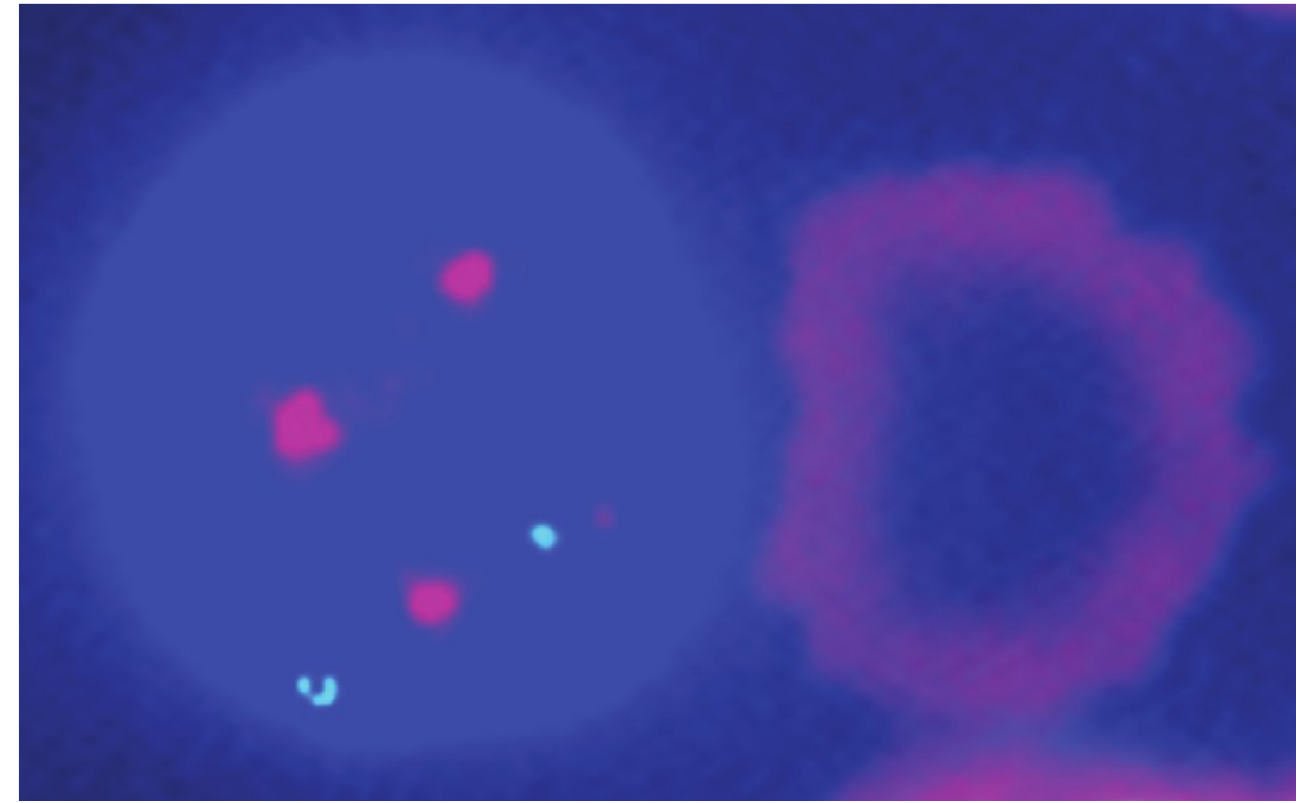


FIGURE 4.3 FISH technique demonstrates trisomy 12 in a case of CLL with prolymphocytoid transformation.

for unbalanced lesions, and allow for detection of copy number-neutral loss of heterozygosity or somatic uniparental disomy (UPD), which cannot be detected by metaphase cytogenetics (19). Genomic array analysis is automated; therefore, results can be objectively and systemically analyzed using biostatistical algorithms. These techniques can be performed on interphase cells, thus even archival specimens can be examined.

There are two major techniques included in this category: the comparative genomic hybridization arrays (CGH-A) and single nucleotide polymorphism arrays (SNP-A) (19). CGH-A rely on the difference in the copy number between differentially labeled test and reference DNA samples. Through competition between test (e.g., tumor) and control diploid DNA, imbalances due to number differences

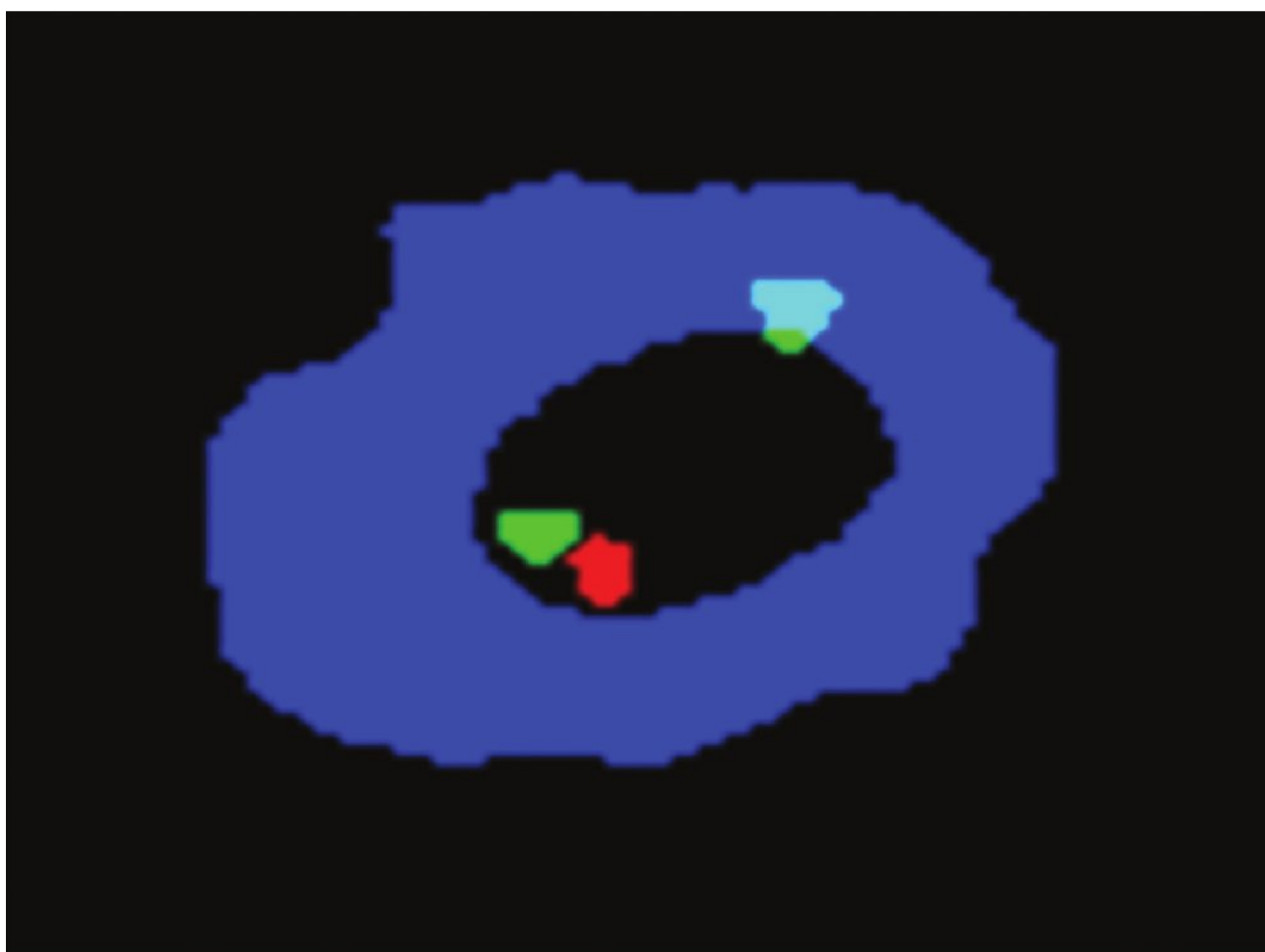


FIGURE 4.2 FISH technique demonstrates the deletion of one retinoblastoma (Rb) gene (single red signal).

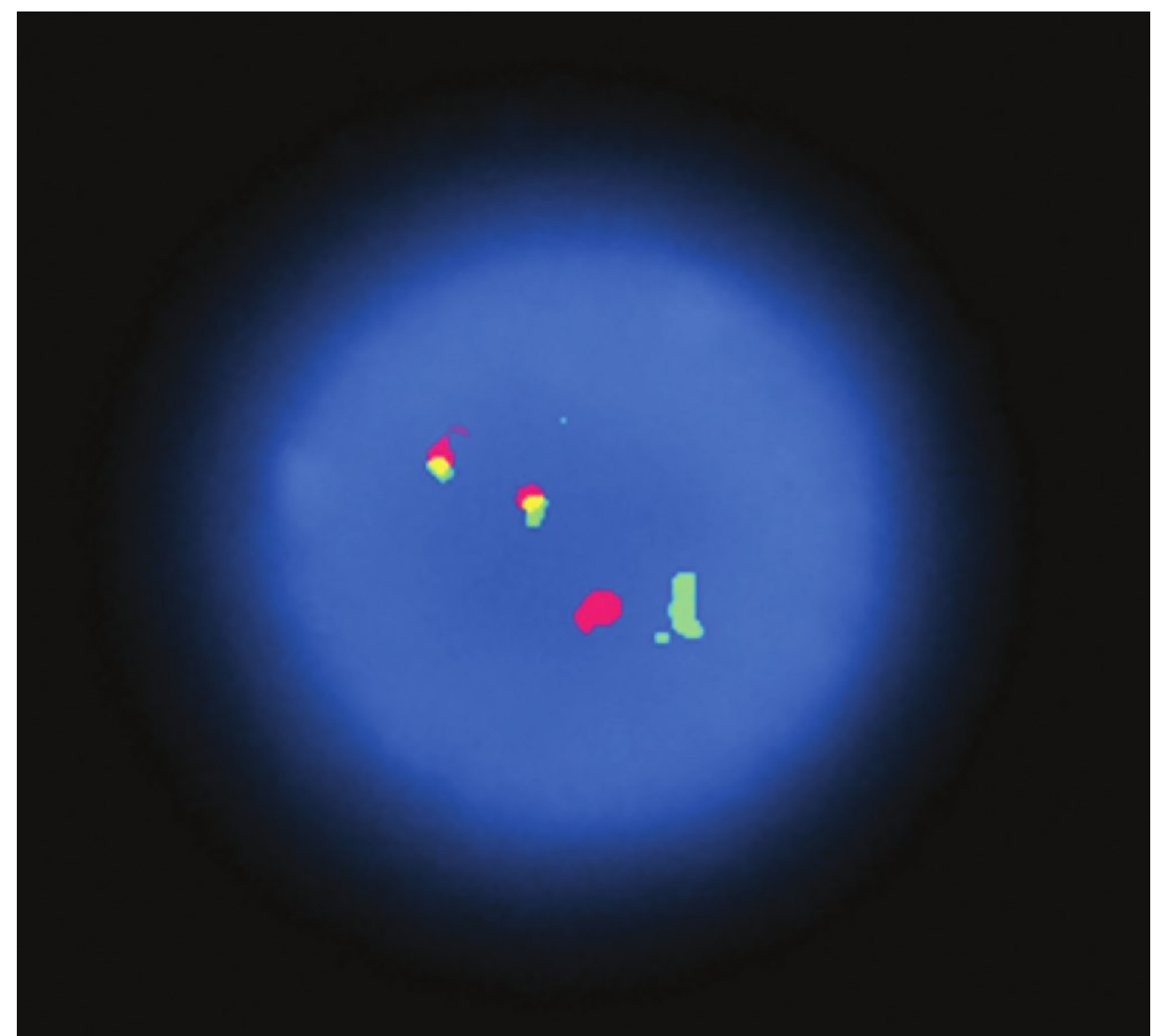


FIGURE 4.4 FISH technique demonstrates double fusion signals (yellow), with one green and one red signal, representing BCL-1/IgH translocation. (From Mary Lowery Nordberg, Ph.D., LSU Health Science Center, Shreveport, LA, with permission.)

result in a shift in the fluorescence spectra. SNP-A rely on oligonucleotide probes corresponding to the allelic variant of selected SNPs. Hybridization of genomic DNA to both probe variants indicates heterozygosity, while a signal for only one allele is consistent with homo/hemizyosity at any given locus.

The overall detection rate of karyotypic aberrations is generally higher by array analysis than metaphase cytogenetics. The most common defects detected by the array techniques are small deletions and gains of chromosomal material, which are not detectable by conventional cytogenetics. Acquired somatic UPD is a new type of chromosomal lesion frequently identified by SNP-A. The first detection of recurrent acquired UPD in hematologic disorders was polycythemia vera, which enabled the subsequent discovery of the JAK2 V617F mutation (20). Since then the array-based karyotyping techniques have been widely applied to various hematologic neoplasms, including plasma cell myeloma, chronic lymphocytic leukemia (CLL), ALL, AML, myelodysplastic syndrome, and myeloproliferative neoplasms (19).

MOLECULAR BIOLOGY

FISH and array-based whole genome scanning technologies can be considered a hybrid of molecular biology and cytogenetics. In addition to FISH, the most commonly used molecular biology techniques in clinical laboratories are Southern blotting and PCR. The variants of PCR include reverse transcriptase PCR (RT-PCR) and quantitative PCR (real-time PCR). In hematology, molecular biology techniques are used to detect rearrangements of the antigen receptor genes (immunoglobulin and T-cell receptor [TCR]) and oncogenes, to identify clonalities of the suspected tumor cells and oncogene translocations. The most current techniques are the analysis of the variable region of immunoglobulin heavy-chain gene (V_H gene) to determine the developmental stage of a particular B-cell tumor and gene expression profiling (GEP), which can stratify lymphomas and leukemias by using a large number of gene probes for the purpose of diagnosis and prediction of therapeutic response as well as prognosis.

Immunoglobulin and T-cell Receptor Gene Rearrangement

Surface immunoglobulin and TCR are the definitive markers of B cells and T cells, respectively. Their production is controlled by a series of genes (21–23). The variable region of the heavy chain of an immunoglobulin molecule is encoded for by three genes, V (variable), D (diversity), and J (joining). The constant region of either heavy chains or light chains is encoded by one gene, C (constant). The heavy-chain (H) gene has about 50 to 100 V_H segments, 25 to 30 D_H segments, and 6 J_H segments (Table 4.1). The κ light-chain gene has about 40 to 80 V_κ segments and 1 J_κ segment. The λ light chain has about 40 V_λ segments and 6 J_λ segments. The light-chain genes have no D segments. All of these segments are arranged in an orderly array on various chromosomes: The heavy-chain gene locus is on 14q32, the κ light-chain locus is on 2p12, and the λ light-chain locus is on 22q11. During the differentiation of the B cell, a series of gene rearrangements takes place (Fig. 4.5).

The heavy-chain gene rearrangement takes place in the pre-B-cell stage when the recombinases encoded by recombination-activating gene (RAG1) and RAG2 genes mediate the random rearrangement of one each of the D and J gene segments. This is followed by the rearrangement of one of the V gene segments. After the completion of the V–D–J rearrangement, the DNA is transcribed into messenger RNA (mRNA) with splicing of the rearranged segments next to the constant (C) region gene. This RNA transcript is then translated into heavy-chain immunoglobulin. The light-chain genes go through the same process and translate into light-chain immunoglobulins at a later stage. The light-chain and heavy-chain immunoglobulins will then combine and form an intact immunoglobulin molecule. The κ light-chain gene is usually rearranged first. If the κ gene rearrangement fails to produce a κ light-chain protein, the λ chain will then be rearranged (24,25).

The TCR is a heterodimer, consisting of two polypeptide chains. Most T cells carry the α – β heterodimer on their cell membrane, whereas some bear the γ – δ receptor (12,26). The α - and δ -chain genes are located on the same locus of chromosome 14. The β - and γ -chain genes are located on the long arm and short arm of chromosome 7, respectively. During normal T-cell development, the d

TABLE 4.1

Characteristics of Receptor Genes

	Heavy chain	κ chain	λ chain	TCR α	TCR β	TCR γ	TCR δ
Locus	14q32	2p12	22q11	14q11	7q34	7p15	14q11
Variable segment	50–100	40–80	40	50–100	75–100	8	4
Diversity segment	25–30	0	0	0	2	0	2
Joining segment	6	5	6	50–100	13	2	3
Constant segment	9	1	6	1	2	2	1

TCR, T-cell receptor.

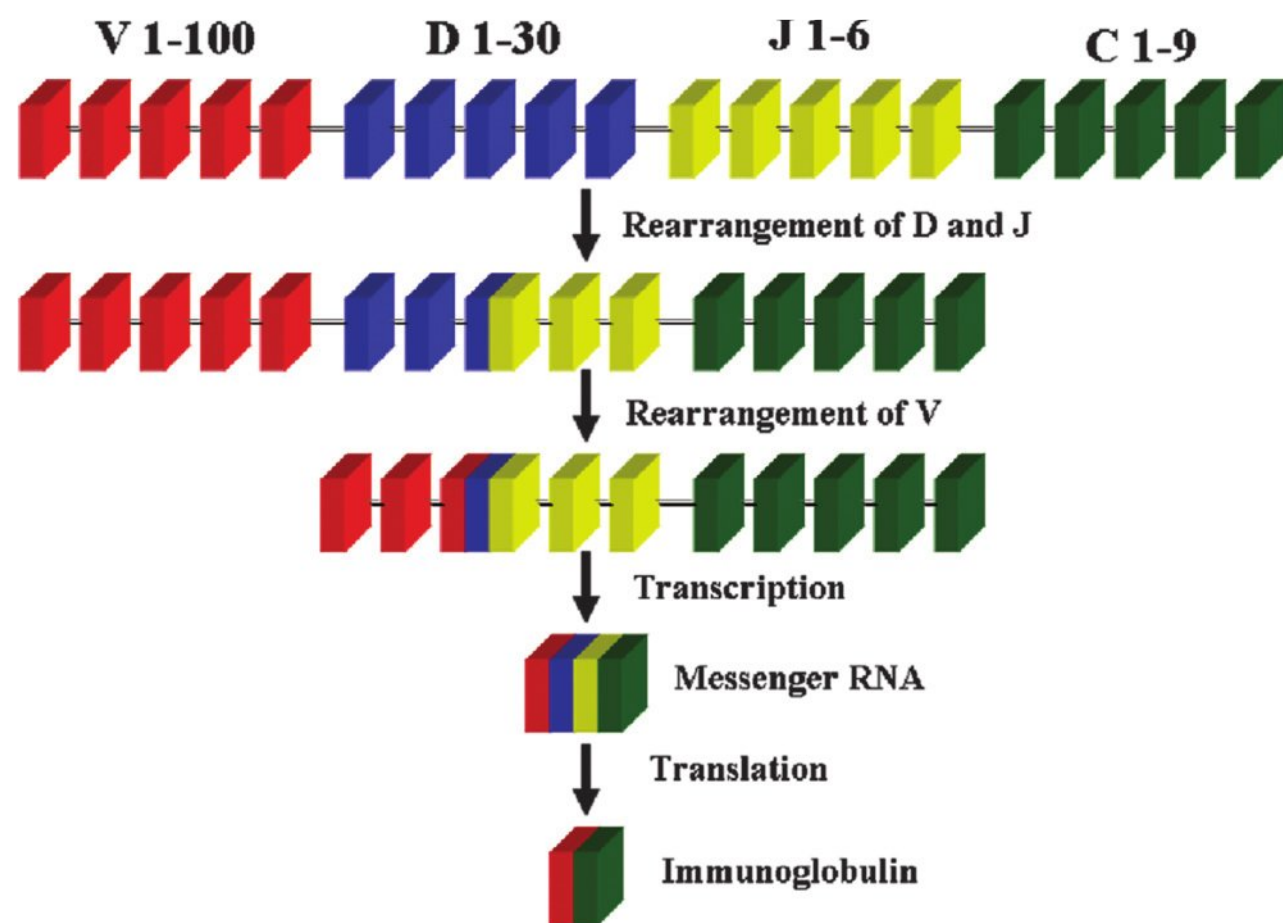


FIGURE 4.5 Immunoglobulin heavy-chain gene rearrangement. The germ line heavy-chain gene is composed of four regions: V (variable), D (diversity), J (joining), and C (constant). Each region contains a certain number of segments. DNA rearrangement is the random selection of one segment from each gene from the germ line to recombine into a new gene. Selected gene segments are transcribed into mRNAs, which are, in turn, translated into proteins (immunoglobulins).

and g chains are probably rearranged at the same time, followed by the b-chain gene. The a-chain gene is the last one to be rearranged.

Somatic Mutation of Immunoglobulin Heavy-Chain Gene

The B lymphocytes that have undergone immunoglobulin gene rearrangement differentiate into mature surface immunoglobulin positive naïve B cells. These naïve B cells circulate in the blood and go to the lymph node, entering the mantle zone and the primary lymphoid follicles (6). After exposure to the antigen, naïve B cells transform into centroblasts and proliferate, forming the germinal center. In the germinal center, somatic mutations occur in the immunoglobulin variable (V_H) region gene. Centroblasts then mature into centrocytes. Centrocytes that have high affinity to the antigen trapped on the processes of

follicular dendritic cells survive, but those with low affinity die by apoptosis. B cells derived from the germinal centers continue to undergo mutation or hypermutation. Somatic mutation of the V_H gene is the hallmark of B cells or tumor cells derived from the germinal center. B cells that do not undergo V_H mutation are called pregerminal center B cells. B cells that undergo V_H mutation but do not have continued mutation show no intraclonal diversity. These cells are called postgerminal center B cells. V_H gene mutation status is determined by DNA sequencing; mutation is defined by <97% homology to germ line.

Southern Blotting

Southern blotting is one of the first nucleic acid probe assays used in clinical molecular laboratories. It is so named because Dr. Southern was the first to use this technique to transfer DNA from the gel onto a filter (27). Subsequently, the same procedure was used to transfer RNA and protein from the gel onto a filter; the new procedures were designated Northern blotting and Western blotting, respectively.

DNA is first isolated from a clinical specimen by phenol-chloroform extraction and ethanol precipitation (27). The DNA extract is then digested by restriction endonucleases, such as BamHI, EcoRI, and HindIII. The resultant restriction fragments are size fractionated by electrophoresis in 0.8% agarose gels. The DNA in the gels is treated with alkali to become single stranded, transferred to a nitrocellulose or nylon membrane, and immobilized by heating. Bands containing DNA of the gene fragment of interest are detected by a DNA probe, usually labeled with a radioisotope, and visualized by autoradiography. Normal lymphocytes show only germ line bands, but clonal lymphocytes reveal one or more bands not identical to the germ lines, which are called rearranged bands or restriction fragment length polymorphism (Fig. 4.6).

It is important to select the appropriate probes for this procedure (27). The C (constant region) probes were first cloned and used as the probes for heavy-chain gene, light-chain gene, and TCR genes. However, the J segment is involved in most rearrangement and is limited in number; therefore, the use of J probes may have a higher positive yield, and these probes have gradually replaced the C probes in all gene rearrangement studies. The higher

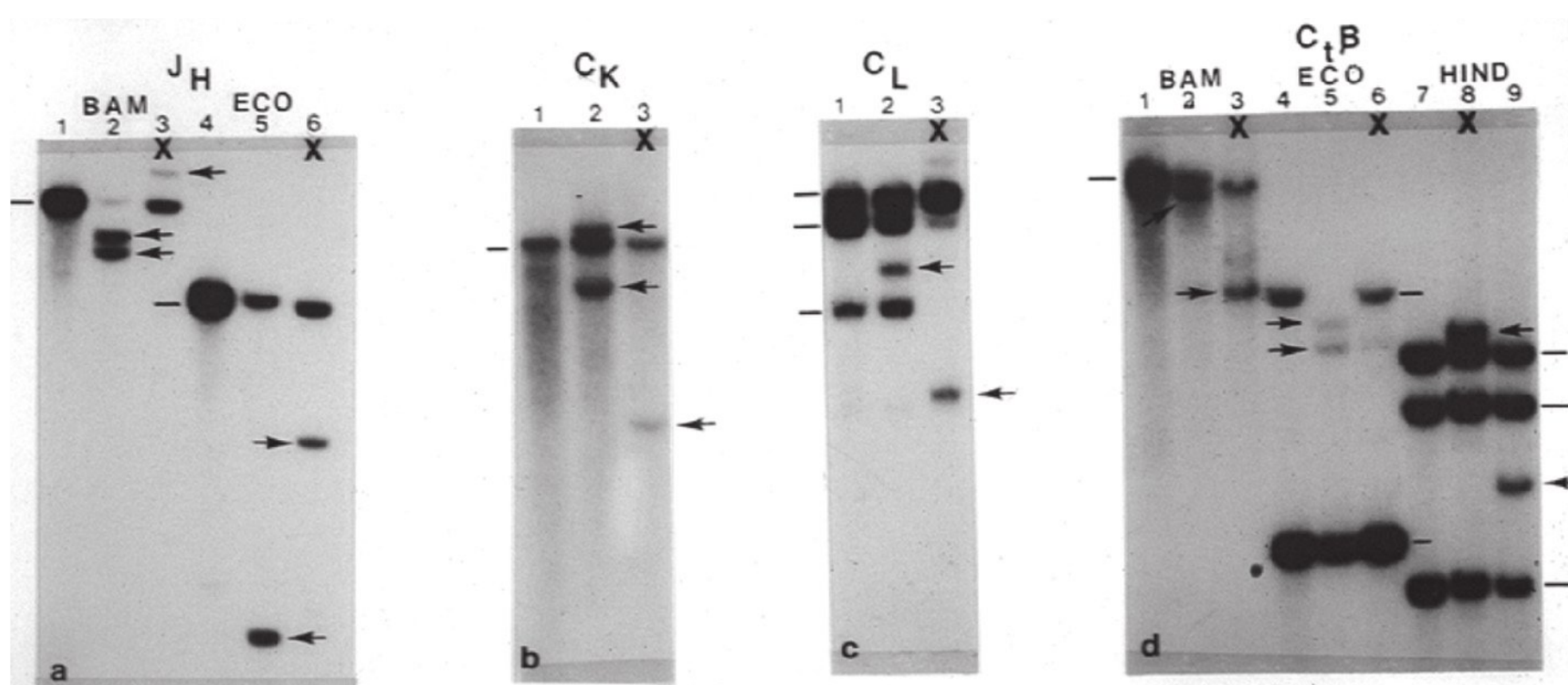


FIGURE 4.6 Southern blotting hybridization analysis of the immunoglobulin heavy-chain gene (J_H), light-chain genes (C_K and C_L), and T-cell receptor b-chain gene (C_b) in a case of B-cell ALL. (From Sun T, et al. Comparison of phenotyping and genotyping of lymphoid neoplasms. *J Clin Lab Anal.* 1989;3:156–162.)

detection rate of a J probe than a C probe is partly due to the fact that C_m is deleted after heavy-chain switch, but J_H remains (28).

For TCR gene analysis, the TCR α -chain gene is composed of an enormous J region; therefore, it is impractical to test the TCR α -chain gene routinely by using either a J probe or C probe. In contrast, the TCR γ -chain gene is difficult to analyze because of its limited number of V segments. As a result, <10 different restriction fragments are generated after the digestion by restriction endonucleases. Therefore, polyclonal T-cell population may produce a visibly rearranged band and will be misinterpreted as a monoclonal band. In addition, this band may sometimes obscure the neoplastic clone. Due to the above reasons, TCR β probe is most frequently used for Southern blotting.

Polymerase Chain Reaction

The Southern blotting technique is time consuming, and sometimes the size of clinical specimens is so minute that it is not sufficient for this sensitive technique. The PCR is an in vitro technique for enzymatic amplification of a DNA segment of interest that can provide sufficient material for Southern blotting. Most frequently, the amplified product is simply subjected to electrophoresis, and the electrophoretogram is stained with a DNA dye, propidium iodide, to demonstrate the rearrangement or germ line bands. Currently, capillary electrophoresis is used. In an automatic instrument, the amplified product is transferred to the capillary loop for electrophoresis. After this procedure, the electrophoretic pattern is scanned by the detector, and a electrophoretogram is generated for interpretation (Fig. 4.7). This technique is superior to gel electrophoresis in identifying the rearrangement bands.

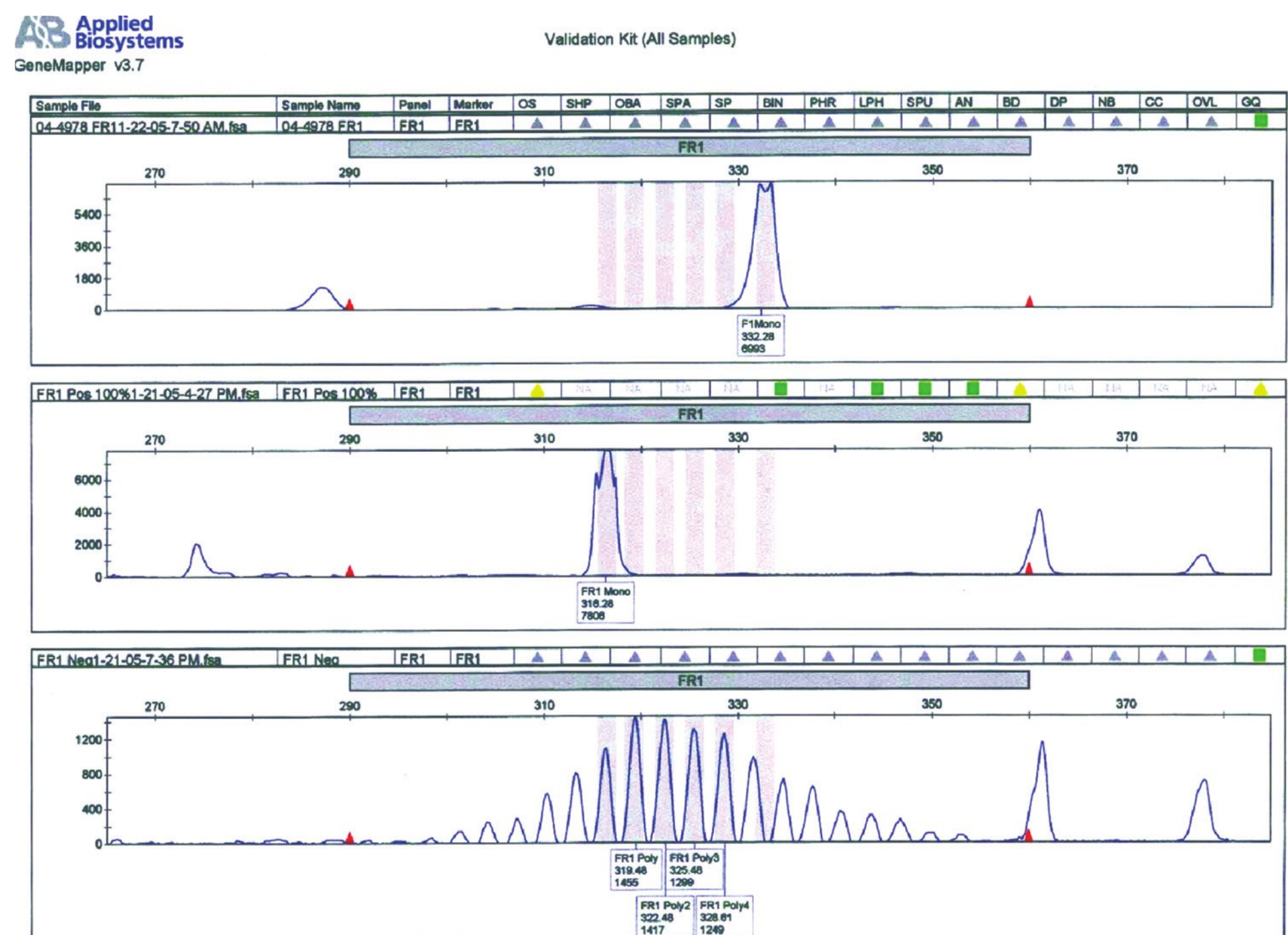
The PCR procedure is composed of repetitive cycling of three simple reactions: DNA denaturation, primer annealing, and primer extension (Fig. 4.8). All reactions take place in the same test tube (well) at various temperatures. DNA denaturation is accomplished by a high temperature (90°C to 95°C), which breaks the hydrogen bonds of the double-stranded DNA and produces single-stranded DNA. Two single-stranded oligonucleotides (primers), synthesized to be complementary to known sequences of the target DNA, are added with polymerases and excess deoxyribonucleoside triphosphates (nucleotides), as the building block for new DNA synthesis. At a lower temperature (45°C to 55°C), the two primers anneal to opposite ends of the two single-stranded DNA molecules derived from the first step. The polymerase then catalyzes the synthesis of a complementary second strand of the new DNA at 72°C, leading to the extension of each annealed primer. Two single-stranded DNA copies are produced in the first cycle, but DNA increases geometrically in subsequent cycles. After repeating the cycle 30 times, about 1 million copies of the target DNA segments are generated in 4 hours.

PCR is most desirable for the diagnosis of lymphomas and leukemias with chromosomal translocation, such as CML (29) and follicular lymphoma (30). Because of its sensitivity, PCR is increasingly frequently used for detection of residual leukemia and lymphoma (31). RT-PCR is used to analyze RNA from tumor cells (28). The first step of this procedure is to copy the mRNA into complementary DNA (cDNA). This procedure is particularly useful for the study of gene expression (32).

Quantitative Polymerase Chain Reaction

Quantitative PCR is mainly used in the field of infectious diseases, but it is also helpful in the detection of minimal

FIGURE 4.7 PCR with capillary electrophoresis technique using three pairs of primers for the detection of immunoglobulin heavy-chain gene rearrangements. Frameworks I and II show a monoclonal peak, representing gene rearrangement. Framework III shows a polyclonal pattern.



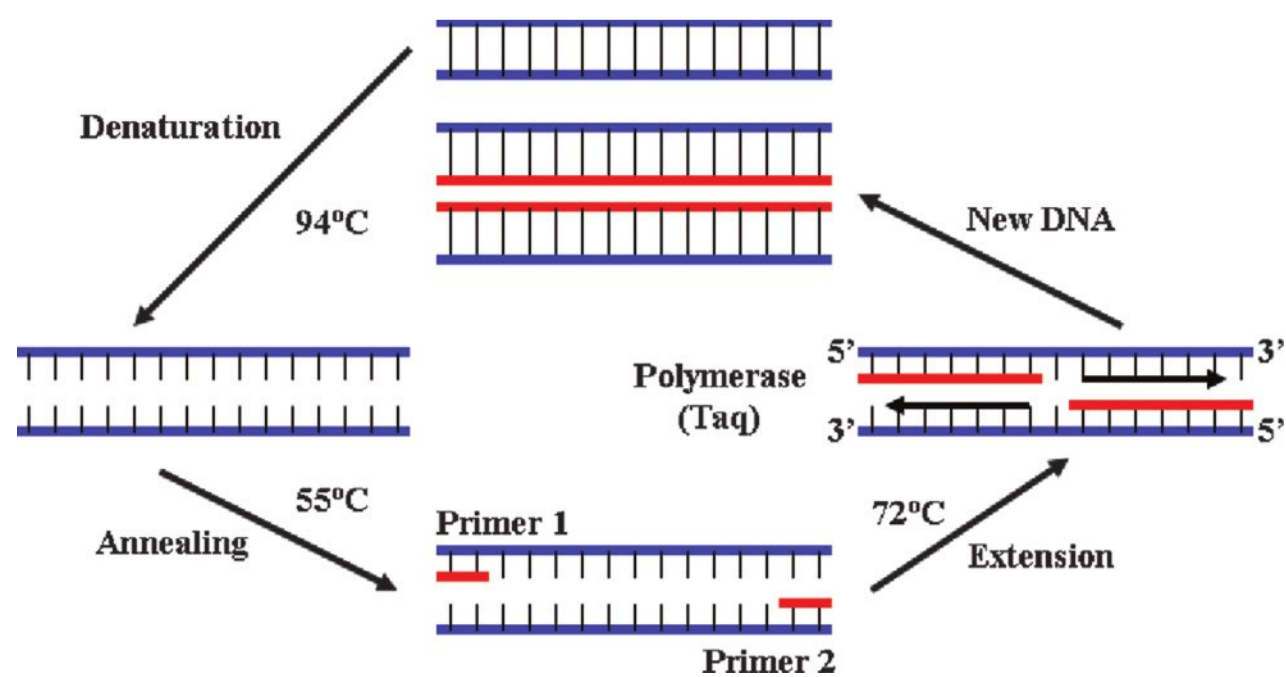


FIGURE 4.8 Scheme of PCR showing the stages of denaturation, annealing, and extension with the reaction of primers and polymerase under different temperatures.

residual disease in different kinds of lymphomas and leukemias (33). The older methodology is competitive PCR, and the current method is real-time quantitative PCR based on the use of fluorogenic probes (34). The fluorogenic probe, also called TaqMan probe, consists of an oligonucleotide to which reporter dye and a quencher dye are attached (Fig. 4.9). When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. During PCR, forward and reverse primers anneal to specific sequences of the target DNA at the 5' and 3' ends. The TaqMan probe anneals to specific sequences in between the forward and reverse primer sites. If hybridization occurs, the probe is cleaved by the 5' nuclease activity of the DNA polymerase. As a result, the quencher dye and reporter dye are separated; the reporter dye then emits its characteristic fluorescence. The ABI Prism Sequence Detection System measures the increase in the reporter dye's fluorescence during the

thermal cycling of the PCR so that a quantitative result is obtained.

Gene Rearrangement in Lymphoproliferative Disorders

Detection of one or more rearranged bands by Southern blotting or PCR techniques indicates a clonal proliferation of lymphocytes so that each of them has the same sized fragments after digestion and these fragments migrate the same distance. The accumulation of DNA from many cells at the same site makes the rearranged band visible with the isotope label. A monoclonal lymphoid population is usually a neoplastic population.

However, there are certain "benign" conditions that show gene rearrangement consistently. Immunoglobulin gene rearrangement has been found in benign lympho-epithelial lesions in Sjögren disease and TCR gene rearrangement in lymphomatoid papulosis (35,36). Gene rearrangement has also been demonstrated in acquired immunodeficiency syndrome (AIDS)-related lymphadenopathy, lymphadenopathy-associated rheumatoid arthritis, posttransplant lymphoproliferative disorders, pityriasis lichenoides et varioliformis acuta, and systemic Castleman disease (12,37). Nevertheless, most of these benign disorders may be premalignant conditions that are susceptible to transformation of neoplasms (24). Malignant transformation of Sjögren disease (35) and AIDS-associated lymphadenopathy (38) are two better known examples.

Another problem in DNA analysis is cross-lineage rearrangements. Rearrangement of TCR was reported in 25% of patients with non-T-cell ALL (39), whereas rearrangement of heavy-chain gene was seen in 10% of patients with T-cell leukemia (40). TCR and immunoglobulin gene rearrangement may also be demonstrated in about 50% of cases

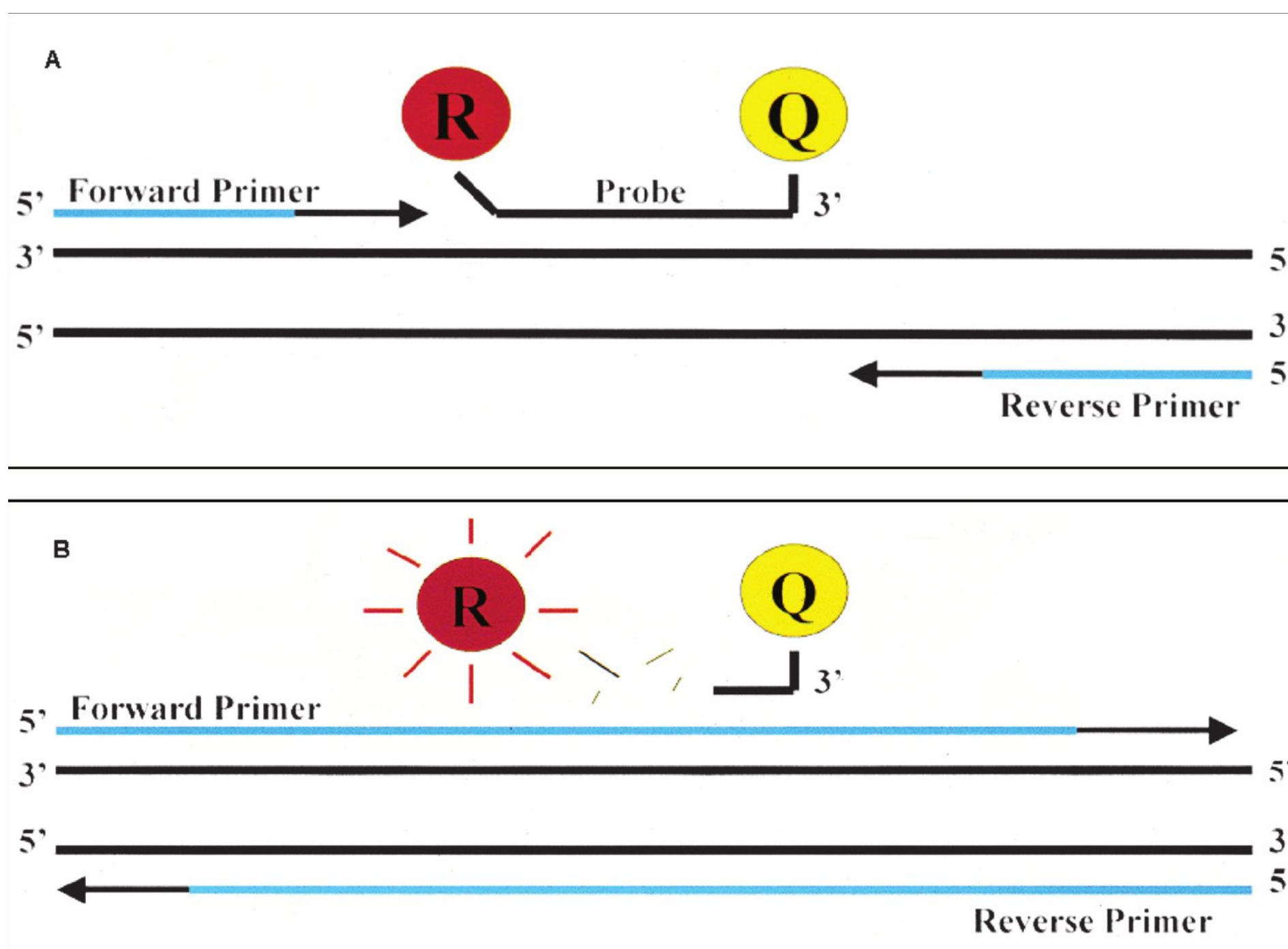


FIGURE 4.9 **A:** The reporter (R) and the quencher (Q) dyes are attached to the probe. When both dyes are attached to the probe, reporter dye emission is quenched. **B:** During the extension, DNA polymerase cleaves the reporter dye from the probe. When separated from the quencher, the reporter dye emits its characteristic fluorescence. The ABI Prism Sequence Detection System measures the increase in the reporter dye's fluorescence during the thermal cycling of the PCR.

of myelogenous leukemia with terminal deoxynucleotidyl transferase (TdT) expression and in 10% without TdT expression (41). However, the rearranged bands in cross-lineage conditions are usually faint and represent partial (DJ segment) rearrangement.

The immunoglobulin light chain is lineage specific; therefore, the demonstration of light-chain rearrangement, regardless of the existence of TCR rearrangement, should be considered to be a B-cell clonal proliferation unless proven otherwise. Immunogenotyping is needed much more for the diagnosis of T-cell neoplasms than for that of B-cell tumors because there are no reliable immunophenotypic markers for the diagnosis of T-cell neoplasms. Unfortunately, the success rate of demonstrating TCR gene rearrangement in T-cell tumors is much lower than that of immunoglobulin gene rearrangement in B-cell malignancies (42,43).

In addition to the diagnosis of lymphoid tumors, gene rearrangement analysis can also identify the clonal origins of two or more coexistent tumors (e.g., composite lymphoma) (44,45) or the relationship of two consecutively appearing tumors (e.g., Richter syndrome) (46,47). Lymphoma cells can also be detected in the peripheral blood by genotyping despite the absence of morphologic evidence of hematogenous spread (48). Furthermore, the DNA hybridization technique can also detect chromosomal translocation by using a probe aiming at the breakpoint

region such as t(9;22) in CML, t(8;14) in Burkitt lymphoma, and t(14;18) in follicular lymphoma.

Oncogenes

Among all the genomic alterations, chromosomal translocation draws most attention. Translocation is frequently a nonrandom change and thus is diagnostic. It also frequently involves oncogenes and antigen receptors in translocation, thus helping to elucidate the mechanism of tumorigenesis, which varies in different tumors (Table 4.2) (49). The mechanism of oncogene activation can be divided as follows:

1. Fusion transcript: The classic example is CML, but the same pattern is encountered in cases of ALL. In these cases, the cytogenetic abnormality is t(9;22)(q34;q11), or the so-called Philadelphia chromosome. The translocation results in the fusion of c-ABL, a protooncogene, on chromosome 9q34 and a restriction region on chromosome 22q11 called the BCR, leading to transcription to an aberrant hybrid c-abl-bcr RNA. The bcr domain activates the tyrosine kinase activity of the c-abl protein (49). The abnormal activity of the tyrosine kinase may disturb the normal process of transduction in the cell and cause malignant transformation.
2. Transcriptional deregulation: The well-known example is Burkitt lymphoma, in which the MYC protooncogene

TABLE 4.2

Chromosomal Translocations in Lymphomas

Neoplasm	Translocation	Genes involved
Anaplastic large cell	t(2;5)(p23;q35)	ALK;NPM
Burkitt	t(8;14)(q24;q32)	c-MYC;IgH
	t(2;8)(p12;q24)	Igκ;c-MYC
	t(8;22)(q24;q11)	c-MYC;Igλ
Burkitt-like*	t(14;18)(q32;q21)	IgH;BCL-2
Cutaneous T cell	t(10;14)(q24;q32)	NFKβ2(LYT-10);IgH
DLBCL	t(3;14)(q27;q32)	BCL-6;IgH
	t(14;15)(q32;q11-13)	IgH;BCL-8
Follicular	t(14;18)(q32;q21)	IgH;BCL-2
Lymphoplasmacytic	t(9;14)(p13;q32)	PAX5 (BSAP);IgH
Mantle cell	t(11;14)(q13;q32)	BCL-1 (CCND1);IgH
Marginal zone/MALT	t(11;18)(q21;q21)	API2;MLT
	t(1;14)(p22;q32)	BCL-10;IgH
Plasma cell myeloma	t(4;14)(p16;q32)	FGFR3;IgH
	t(14;16)(q32;q23)	IgH;c-MAF
	t(16;22)(q23;q11)	c-MAF;Igλ
Small lymphocytic/CLL	t(14;19)(q32;q13)	IgH;BCL-3

*The new terminology in 2008 WHO classification is B-cell lymphoma, unclassifiable with features intermediate between DLBCL and Burkitt lymphoma.

CLL, chronic lymphocytic leukemia; MALT, mucosa-associated lymphoid tissue.

is translocated from chromosome 8 to chromosome 14 and juxtaposed with the heavy-chain gene. As a result of the translocation, MYC submits to the control of the transcriptional enhancer of the immunoglobulin gene and is thus activated or deregulated. Constitutive MYC expression may prevent cells from entering the resting state (G_0 phase) and differentiating, leading to continuing proliferation of undifferentiated cells (50).

3. BCL-2 overexpression: Follicular lymphoma is characterized by the genomic alteration of t(14;18), in which the protooncogene BCL-2 (18q12) moves into the proximity of the immunoglobulin heavy-chain enhancer region (14q32). As a result, the protooncogene is activated (deregulated) and the functional BCL-2-Ig fusion protein is overexpressed. The BCL-2 gene encodes for an inner mitochondrial membrane protein that plays a role in blocking programmed cell death (apoptosis) (51). Therefore, cells with abnormal expression of this protein remain in the G_0 phase and become immortalized.
4. PRAD1 overexpression: In MCL, the protooncogene BCL-1 (11q13) is juxtaposed to an immunoglobulin enhancer sequence located on chromosome 14. This translocation results in deregulation of the PRAD1 gene linked to the BCL-1 locus (52). PRAD1 encodes for cyclin D1, a cell cycle protein. As a result of PRAD1 activation, the G_1 -S transition of the cell cycle is disturbed and the t(11;14)-carrying cells cannot exit from the cell cycle, leading to an expanded B-cell department (53).
5. Activation by point mutation or gene amplification: Mutation of the RAS genes has been found in some hematologic neoplasms, including AML, CML, ALL, and plasma cell myeloma (17). Amplification of the n-MYC gene has been demonstrated in neuroblastoma and the NEU gene in breast and ovarian carcinoma (49), but this mechanism of activation has not been detected in lymphoid neoplasms.
6. Deletion or mutation of tumor suppressor genes: Tumor suppressor genes, such as p53, have been reported to be involved in the tumorigenesis of Burkitt lymphoma and Richter transformation (54).

Gene Expression Profiling

GEP using DNA microarrays has great potential in the fields of diagnosis, prediction of prognosis, and guidance of treatment for hematologic neoplasms (55–57). This technique is used to tether hundreds or thousands of gene-specific probes in arrays on a solid phase, such as glass. RNA is extracted from tissues of interest, labeled with a detectable marker (usually fluorochromes). The samples containing this mRNA are then hybridized with the gene-specific probes on the array. Images are generated by the use of confocal laser scanning, and the relative fluorescence intensity of each gene-specific probe represents the level of expression of the particular gene.

After the expression data are collected, they are presented in a matrix in which each row represents a particular gene and each column represents a specific tissue sample. To facilitate data interpretation, elements of the data in a matrix are often rendered in color to indicate the level of expression of each gene in each sample. The colors

used are based on the log ratio for each sample measured as compared with a control sample. For instance, log ratio values close to zero are represented by black, those with values >0 are represented by red (indicating up-regulated genes), and those with negative values are represented by green (indicating down-regulated genes). The original expression matrix generally shows no apparent pattern or order. Programs that perform clustering are required to arrange the rows and columns in order.

There are two methods for analysis of data. The supervised method depends on prior knowledge about the sample to search for genes that correlate with a disease state. The unsupervised method disregards prior knowledge of the nature of the specimens, is often used for screening, and divides the data into clusters with either the hierarchical clustering or k-mean cluster analysis.

After data analysis, gene expression signatures can be recognized. A gene expression signature is defined as a group of genes that is characteristically expressed in a particular group of cells belonging to a certain cell lineage, disease entity, or subtype of leukemia and/or lymphoma.

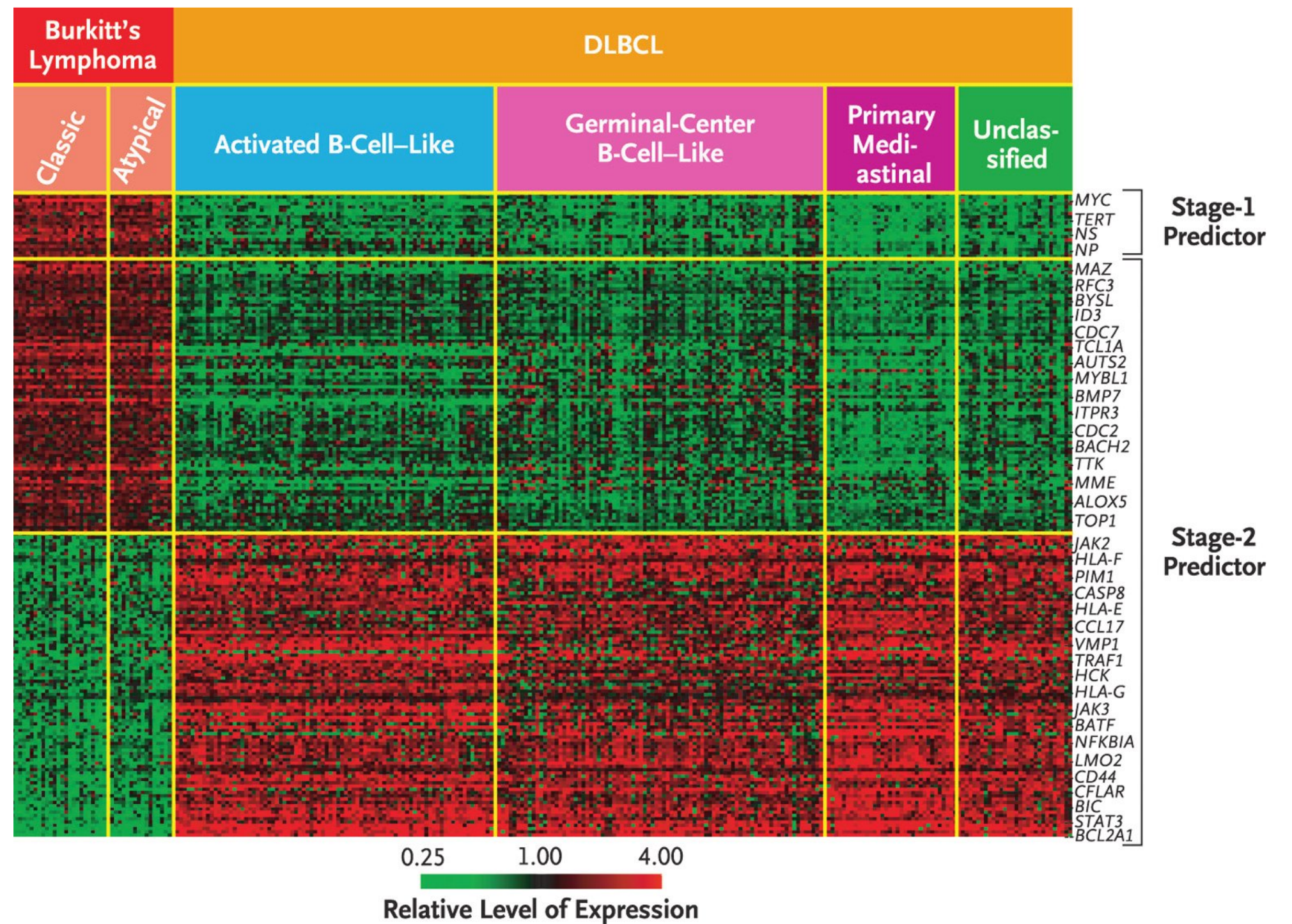
The usefulness of GEP is exemplified by the studies of diffuse large B-cell lymphoma (DLBCL), CLL, and Burkitt lymphoma. Approximately 40% of DLBCL patients can be cured, whereas the remaining patients die within a few years despite treatment. These two groups of patients can now be stratified with GEP. The former group shows the germinal center B cell-like (GCB) signature, whereas the latter group, the activated B cell-like (ABC) signature (58). Furthermore, the ABC group (but not the GCB group) reveals a high expression profile of nuclear factor-kappa B (NF- κ B) target genes. A new compound, PS-341, may inhibit NF- κ B, so that it is potentially a powerful therapeutic agent in combination with other chemotherapeutic regimens (59). In DLBCL cases transformed from follicular lymphoma, almost all were of the GCB subtype, as identified by GEP (60).

CLL patients can also be divided into two prognostic groups by GEP. The major difference between the two groups is the presence or absence of V_H gene somatic mutation. Cases with unmutated CLL cells represent a naïve B-cell origin, demonstrating a worse prognosis than those with mutations (61). For CLL patients with the V_H mutation subtype, a treatment with cytotoxic drugs should be postponed until the appearance of clinical progression (56).

The distinction between Burkitt lymphoma and DLBCL is sometimes difficult to determine by using morphologic and immunologic criteria, yet it is important to distinguish these two entities because their treatment and prognosis are quite different. Cytogenetic studies are helpful because Burkitt lymphoma has c-MYC rearrangement, but DLBCL may have BCL-2 rearrangement. However, two recent studies with GEP showed that even cytogenetics is not entirely reliable: Burkitt lymphoma may not have c-MYC or may have BCL-2 rearrangement, and DLBCL may have c-MYC gene rearrangement (62,63). Therefore, GEP is the only reliable means to make such an important distinction (Fig. 4.10).

Identification of cyclin D1 has been considered a gold standard for the diagnosis of MCL. However, an increasing number of cyclin D1-negative MCL cases have been found recently (60). These cases even lack the characteristic

FIGURE 4.10 GEP of Burkitt lymphoma and DLBCL shows distinctly different signatures. (Reproduced from Dave SS, et al. Molecular diagnosis of Burkitt's lymphoma. *N Engl J Med.* 2006;354:2435 with permission.)



translocation, t(11;14) by FISH. Nevertheless, these cases show characteristic morphology and similar clinical features as cyclin D1-positive MCL. These aberrant MCL cases can only be identified by GEP with up-regulation of cyclin D2 or D3 (64).

In an outcome-based analysis with GEP on 191 cases of follicular lymphoma, two principal signatures, namely immune response 1 (IR-1) and immune response 2 (IR-2) were identified (65). Neither was detected in the malignant cell fraction. Instead, the IR-1 signature was highly expressed in T cells, and the IR-2 signature in monocytes and macrophages. These findings indicated that both prognostic signatures reflect nonmalignant immune cells within the biopsy specimens and that the microenvironment determines the prognosis.

Tumorigenesis and Signal Transduction Pathways

Cells have evolved mechanisms to sense environmental stimuli and to respond to them appropriately. The human cells must respond to a great diversity of signals, including nutrients, growth factors, hormones, neurotransmitters, and sensory input (66,67). These signals affect the growth state of cells by mobilizing resting cell populations into active growth and telling proliferating cells to cease growth. To achieve these functions, the primary signal from a cytokine must be received by the receptor on the cell surface, transmitted across the cell (plasma) membrane, and in some cases, transmitted across the nuclear membrane as well.

The signal transduction pathways involve a series of biochemical reactions, mainly through the intracellular protein kinases (e.g., tyrosine, serine and threonine kinases) to phosphorylate, thus activate, specific proteins (68).

The most important proteins in this system are the DNA-binding proteins called transcription factors. The intracellular pathways, by which cytokines activate transcription factors, are diverse, and they play an important role in our physiologic functions as well as in pathogenesis of many diseases, especially cancers. This process may sometimes involve second messengers, such as cAMP, which, through transcription factor phosphorylation, may reach the target gene in the nucleus (66,67). Gene transcription, finally, induces many physiologic and pathologic effects. The signal transduction pathways usually involve a cytosolic phase and a nuclear phase, but some pathways may not have a nuclear phase.

Oncogenes lie along the signaling pathways by which cells receive and execute growth instructions (66,67). Oncogenes are activated by mutations. A structural mutation leads to the constitutive activity of a protein without an incoming signal (e.g., the protein kinases and RAS). A regulatory mutation, in contrast, leads to expression of the gene at the wrong place or time (e.g., the nuclear oncogene and the growth factors) (66,67).

Many gene/oncogene products involving in tumorigenesis, such as BCR-ABL1, KIT, JAK2, and FLT3, are tyrosine kinases, and thus become potential targets for the treatment with tyrosine kinase inhibitors. For instance, the inhibitor of BCR-ABL1 with imatinib has been used successfully in the treatment of chronic myeloid leukemia.

Alteration of signaling pathway has been discovered increasingly in various kinds of lymphomas and leukemias. For instance, NOTCH1 signaling plays an important role in the pathogenesis of T-cell lymphoblastic leukemia, and NOTCH1 inhibitor (such as gsecretase inhibitor) may prove to be effective for the treatment of this entity (69). Aberration of core-binding factor (CBF) transcription

factor pathway is involved in AML with t(8;21)(q22;q22) and inv(16)(p13.1;q22) or t(16;16)(p13.1;q22) (70,71).

Epigenetics in Tumorigenesis

In recent years, it has been found that tumorigenesis is not only induced by cytogenetics but also frequently caused by epigenetics. Epigenetics is defined by stable gene alterations in gene expression with no underlying modifications in the gene sequence (72). The best example is cell differentiation, in which multiple cell types diverge physiologically despite a common genetic code (72). Epigenetic changes are heritable through mitosis and potentially meiosis. There are three general molecular mechanisms carrying epigenetic information, which are DNA methylation, histone modifications, and small noncoding RNA interference. DNA methylation leads to silencing of genes by direct inhibition of transcription factor binding to their relative sites and by recruitment of methyl-binding domain proteins (MBDs). These MBDs are present in transcription corepressor complexes involving several other members of the epigenetic machinery, such as histone deacetylases and histone methyltransferases, resulting in chromosome reconfiguration and gene silencing.

Histones are small proteins that form a core around which DNA is wrapped, forming nucleosomes. Nucleosomes are the basic in vivo structural unit of DNA and consist of eight histone molecules around which a loop of DNA is wrapped (73). The posttranslational histone modifications include acetylation, methylation, phosphorylation, ubiquitination, sumoylation, and ADP ribosylation. These modifications may play the same role as DNA methylation.

Small noncoding RNA interference is represented by microRNA(miRNA) alterations. miRNAs are single-stranded RNA molecules of 20 to 23 nucleotide length that control gene expression in many cellular processes (74). These molecules typically reduce the stability of mRNAs, including those of genes that mediate process in tumorigenesis. Therefore, miRNA dysregulation may induce formation of neoplasms either through the role as tumor suppressors or oncogenes. miRNAs that are up- or down-regulated in malignancies are respectively referred to as oncogenic or tumor-suppressor miRNAs.

In core-binding factor leukemias (CBFLs), the CBFL-associated fusion proteins may lead to up-regulation of the KIT receptor by down-regulation of microRNA (miR) 222/221 (75). In contrast, miR125b-2 is a positive regulator of megakaryopoiesis and an oncomiR involved in the pathogenesis of trisomy 21-associated megakaryocytic leukemia (76). In Sézary syndrome, miR-223 is down-regulated, which distinguishes it from mycosis fungoides and from healthy controls (77).

REFERENCES

1. Bagg A. Clinical applications of molecular genetic testing in hematologic malignancies: advantages and limitations. *Hum Pathol.* 2003;34:352–358.
2. Müller-Hermelink HK. Genetic and molecular genetic studies in the diagnosis of B-cell lymphomas: marginal zone lymphomas. *Hum Pathol.* 2003;34:336–340.
3. Chan WC, Hans CP. Genetic and molecular genetic studies in the diagnosis of T and NK cell neoplasia. *Hum Pathol.* 2003;34:314321.
4. Cook JR, Shekhter-Levin S, Swerdlow SH. Utility of routine classical cytogenetic studies in the evaluation of suspected lymphomas. *Am J Clin Pathol.* 2004;121:826–835.
5. Kiechle FL, Zhang X, Holland C. Molecular pathology: future issues. *Arch Pathol Lab Med.* 2006;130:650–653.
6. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008.
7. Anagnostopoulos I, Dallenback F, Stein H. Diffuse large cell lymphomas. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:855–913.
8. Sun T, Nordberg ML, Cotelingam JD, et al. Fluorescence in situ hybridization: method of choice for a definitive diagnosis of mantle cell lymphoma. *Am J Hematol.* 2003;74:78–84.
9. Lida S, Rao PH, Ueda R, et al. Chromosomal rearrangement of the PAX-5 locus in lymphoplasmacytic lymphoma with t(9;14)(p13;q32). *Leuk Lymphoma.* 1999;34:25–33.
10. Le Beau MM. Role of cytogenetics in the diagnosis and classification of hematopoietic neoplasms. In: Knowles DM, ed. *Neoplastic Hematopathology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:391–418.
11. Dewald GW, Ketterling RP, Wyatt WA, et al. Cytogenetic studies in neoplastic hematologic disorders. In: McClatchey KD, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:658–685.
12. Griesser H, Takchuk D, Reis MD, et al. Gene rearrangements and translocations in lymphoproliferative diseases. *Blood.* 1989;73:1402–1415.
13. Pui CH, Christ WM, Look AT. Biology and clinical significance of cytogenetic abnormalities in childhood acute lymphoblastic leukemia. *Blood.* 1990;76:1449–1463.
14. Kristoffersson U, Heim S, Mandahl N, et al. Prognostic implications of cytogenetic findings in 106 patients with non-Hodgkin's lymphoma. *Cancer Genet Cytogenet.* 1987;25:55–64.
15. Schouten HC, Sanger WG, Weisenburger DD, et al. Chromosomal abnormalities in untreated patients with non-Hodgkin's lymphoma. Associations with histology, clinical characteristics and treatment outcome. *Blood.* 1990;75:1841–1847.
16. Sun T, Eisenberg A, Ben P, et al. Comparison of phenotyping and genotyping of lymphoid neoplasms. *J Clin Lab Anal.* 1989;3:156–162.
17. Dewald GW, Noel P, Dahl RJ, et al. Chromosome abnormalities in malignant hematologic disorders. *Mayo Clin Proc.* 1985;60:675–689.
18. Kluin PM, Schuurin E. FISH and related techniques in the diagnosis of lymphoma. *Cancer Surv.* 1997;30:3–20.
19. Maciejewski JP, Tiu RV, O'Keefe C. Application of array-based whole genome scanning technologies as a cytogenetic tool in hematological malignancies. *Br J Haematol.* 2009;146:479–488.
20. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol.* 2002;30:229–236.
21. Delves PJ, Roitt IM. The immune system: first of two parts. *N Engl J Med.* 2000;343:37–49.
22. Jung D, Giallourakis C, Mostoslavsky R, et al. Mechanism and control of V(D)J recombination at the immunoglobulin heavy chain locus. *Annu Rev Immunol.* 2006;24:541–570.
23. Alkan S, Hanson CA. Clinical applications of molecular biology hematopoietic disorders. In: McClatchey KD, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:211–240.

24. Sklar J, Weiss LM. Applications of antigen receptor gene rearrangements to the diagnosis and characterization of lymphoid neoplasms. *Annu Rev Med*. 1988;39:315–334.
25. Wilman CL, Griffith BB, Whittaker M. Molecular genetic approaches for the diagnosis of clonality in lymphoid neoplasms. *Clin Lab Med*. 1990;10:119–149.
26. Davis MM, Bjorkman PJ. T-cell receptor genes and T-cell recognition. *Nature*. 1988;334:395–402.
27. Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol*. 1975;98:503–517.
28. Cossman J, Fend F, Staudt I, et al. Application of molecular genetics to the diagnosis and classification of malignant lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:365–390.
29. Dobrovic A, Trainor KJ, Morley AA. Detection of the molecular abnormality in chronic myeloid leukemia by use of the polymerase chain reaction. *Blood*. 1988;72:2063–2065.
30. Lee MS, Chang KS, Cabamillas F, et al. Detection of minimal residual cell carrying the t(14;18) by DNA sequence amplification. *Science*. 1987;237:175–178.
31. Negrin RS, Blume KG. The use of polymerase chain reaction for the detection of minimal residual malignant disease. *Blood*. 1991;78:255–258.
32. Ferreira-Conzalez A, Buller AM, Barkus ME, et al. Introduction to molecular diagnostics. In: McClatchey KD, ed. *Clinical Laboratory Medicine*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:145–162.
33. Gabert J. Detection of recurrent translocations using real time PCR: assessment of the technique for diagnosis and detection of minimal residual disease. *Hematologica*. 1999;84:107–109.
34. Orlando C, Pinzani P, Pazzagli M. Development in quantitative PCR. *Clin Chem Lab Med*. 1998;36:255–269.
35. Hyjeh E, Smith WJ, Isaacson PG. Primary B-cell lymphoma of salivary glands and its relationship to myoepithelial sialadenitis. *Hum Pathol*. 1988;19:766–776.
36. Weiss LM, Wood GS, Trela M, et al. Clonal T-cell populations in lymphomatoid papulosis. Evidence of lymphoproliferative origin for clinically benign disease. *N Engl J Med*. 1986;315:475–479.
37. Waldmann TA. The rearrangement of immunoglobulin and T-cell receptor genes in human lymphoproliferative disorders. *Adv Immunol*. 1987;40:247–313.
38. Levy N, Nelson J, Meyer P, et al. Reactive lymphoid hyperplasia with single class (monoclonal) surface immunoglobulin. *Am J Clin Pathol*. 1983;80:300–308.
39. Tawa A, Hozumi N, Minden M, et al. Rearrangement of the T-cell receptor b-chain gene in non-T-cell, non-B-cell acute lymphoblastic leukemia of childhood. *N Engl J Med*. 1985;313:1033–1037.
40. Kuchingham GR, Rovigatti U, Maueer AM, et al. Rearrangements of immunoglobulin heavy chain genes in T-cell acute lymphoblastic leukemia. *Blood*. 1985;65:725–729.
41. Seremetis SV, Pelicci PG, Tabilio A, et al. High frequency of clonal immunoglobulin on T-cell receptor gene rearrangements in acute myelogenous leukemia expressing terminal deoxynucleotidyl transferase. *J Exp Med*. 1987;165:1703–1712.
42. Weiss LM, Picker LJ, Grogan TM, et al. Absence of clonal beta and gamma T-cell receptor gene rearrangements in a subset of peripheral T-cell lymphomas. *Am J Pathol*. 1988;130:436–442.
43. O'Connor NTJ, Wainscoat JS, Weatherall DJ, et al. Rearrangement of the T-cell receptor d-chain in the diagnosis of lymphoproliferative disorders. *Lancet*. 1985;1:1295–1297.
44. Sklar J, Cleary ML, Theilemans K, et al. Biclinal B-cell lymphoma. *N Engl J Med*. 1984;311:20–27.
45. Sun T, Susin M, Koduru P, et al. Immunophenotyping and immunogenotyping of composite lymphoma with Ki-1 component. *Hematol Pathol*. 1992;6:179–192.
46. Sun T, Susin M, Desner M, et al. The clonal origin of two cell populations in Richter's syndrome. *Hum Pathol*. 1990;21:722–728.
47. Nakamine H, Masih AS, Sanger WG, et al. Richter's syndrome with different immunoglobulin light chain types. Molecular and cytogenetic features indicate a common clonal origin. *Am J Clin Pathol*. 1992;97:656–663.
48. Horning SJ, Galili N, Cleary M, et al. Detection of non-Hodgkin's lymphoma in the peripheral blood by analysis of antigen receptor gene rearrangements. Results of a prospective study. *Blood*. 1990;75:1139–1145.
49. Tam W, Dall-Favera R. Protooncogenes and tumor suppressor genes in hematopoietic malignancies. In: Knowles DM, ed. *Neoplastic Hematopathology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:329–364.
50. McKeithan TW. Molecular biology of non-Hodgkin's lymphoma. *Semin Oncol*. 1990;17:30–42.
51. Hockenberry D, Nunez G, Millman C, et al. Bcl-2 in an inner-mitochondrial membrane protein that blocks programmed cell death. *Nature*. 1990;348:334–336.
52. Rosenberg CL, Wong E, Petty E, et al. PRAD1, a candidate BCL-1 oncogene: mapping and expression in centrocytic lymphoma. *Proc Natl Acad Sci U S A*. 1991;88:9638–9642.
53. Rimokh R, Berger R, Delso G, et al. Detection of the chromosomal translocation t(11;14) by polymerase chain reaction in mantle cell lymphomas. *Blood*. 1994;83:1871–1875.
54. Gaidano G, Ballerini P, Gong JZ, et al. p53 mutations in human lymphoid malignancies: association with Burkitt's lymphoma and chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*. 1991;88:5413–5417.
55. Quackenbush J. Microarray analysis and tumor classification. *N Engl J Med*. 2006;354:2463–2472.
56. Davis RE, Staudt LM. Molecular diagnosis of lymphoid malignancies by gene expression profiling. *Curr Opin Hematol*. 2002;9:333–338.
57. Dunphy CH. Gene expression profiling data in lymphoma and leukemia: review of the literature and extrapolation of pertinent clinical applications. *Arch Pathol Lab Med*. 2006;130:483–520.
58. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503–511.
59. Adams J. Proteasome inhibition in cancer: development of PS-341. *Semin Oncol*. 2001;28:613–619.
60. Orsborne C, Byers R. Impact of gene expression profiling in lymphoma diagnosis and prognosis. *Histopathology*. 2011;58:106–127.
61. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848–1854.
62. Hummel M, Bentink S, Berger H, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *N Engl J Med*. 2006;354:2419–2430.
63. Dave SS, Fu K, Wright GW, et al. Molecular diagnosis of Burkitt lymphoma. *N Engl J Med*. 2006;354:2431–2442.
64. Fu K, Weisenburger DD, Greiner TC, et al. Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. *Blood*. 2005;106:4315–4321.
65. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med*. 2004;351:2159–2169.



66. Watson JD, Gilman M, Witkowski J, et al. Recombinant DNA. 2nd ed. New York, NY: W.H. Freeman and Co.; 1992.
67. Watson JD, Cady AA, Myers RM, et al. Recombinant DNA, Genes and Genomes—A Short Course. 3rd ed. New York, NY: W.H. Freeman; 2007.
68. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 7th ed. Philadelphia, PA: Elsevier; 2011.
69. Pear WS, Aster JC. T-cell acute lymphoblastic leukemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. *Curr Opin Hematol*. 2004;11:426–433.
70. Peterson LF, Zhang DE. The 8;21 translocation in leukemogenesis. *Oncogene*. 2004;23:4255–4262.
71. Liu PP, Hajra A, Wijmenga C, et al. Molecular pathogenesis of the chromosome 16 inversion in the M4EO subtype of acute myeloid leukemia. *Blood*. 1995;85:2289–2302.
72. Taby R, Issa J-PJ. Cancer epigenetics. *CA Cancer J Clin*. 2010;60:376–392.
73. Issa J-P. Epigenetic changes in the myelodysplastic syndrome. *Hematol Oncol Clin N Am*. 2010;24:317–330.
74. Faraji TA, Spitzer JL, Morozov P, et al. miRNA in human cancer. *J Pathol*. 2011;223:102–115.
75. Brioschi M, Fischer J, Cairoli R, et al. Down-regulation of microRNAs 222/221 in acute myelogenous leukemia with deranged core-binding factor subunits. *Neoplasia*. 2010;12:866–876.
76. Klusmann J-H, Li Z, Böhmer K, et al. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. *Genes Dev*. 2010;24:478–490.
77. Ballabio E, Mitchell T, van Kester MS, et al. MicroRNA expression in Sézary syndrome: identification, function, and diagnostic potential. *Blood*. 2010;116:1105–1113.

Classification of Hematologic Neoplasms

There are two major groups of hematologic neoplasms: lymphoma and leukemia. Lymphoma is lymphoid tumors initially confined to lymphoid organs or extranodal tissue, whereas leukemia includes lymphoid and myeloid neoplasms originating from the bone marrow and circulating in the peripheral blood. However, with the advent of new technology, especially immunophenotyping and molecular biology, lymphoma cells can be detected in blood and bone marrow even in the relatively early stage, and the demarcation between lymphoma and leukemia is sometimes blurred. Lymphoma and leukemia have their counterparts, such as lymphoblastic lymphoma versus acute lymphoblastic leukemia (ALL) and small lymphocytic lymphoma (SLL) versus chronic lymphocytic leukemia (CLL). The tumor cells of the counterparts may have the same morphology, immunophenotype, immunogenotype, karyotype, and clinical characteristics, such as the presence of a mediastinal mass in both lymphoblastic lymphoma and ALL of T-cell origin. It has recently been found that the difference in location (tissue vs. blood) between lymphoma and leukemia may be associated with the presence or absence of adhesion molecules or lymphokine receptors on the tumor cells. For instance, the difference in phenotype between SLL and CLL is the presence of the adhesion molecule, CD11a/CD18 (LFA-1), on SLL cells (1). In contrast, CLL cells express the lymphokine receptors, CXCR4 and CCR7, which are not present on the SLL cells (2). The high frequency of the leukemic phase in Burkitt lymphoma is due to its lack of LFA-1 (3).

Leukemia can be further divided into acute and chronic groups. In acute leukemias, the clinical course is rapidly progressive, and the leukemic cells are immature blasts. Chronic leukemias are just the opposite: The clinical course is slow and indolent, and the leukemic cells are mature appearing in lymphoid leukemia or intermediate forms (promyelocytes, myelocytes, and metamyelocytes) in myeloid leukemia. Although lymphoma is not divided into acute and chronic forms, lymphoma cell types can also be differentiated on the basis of maturation. For instance, T-cell lymphoma can be divided into thymic and postthymic (peripheral T cell) subtypes. The homogeneity of leukemia and lymphoma cells in terms of their maturation stage prompted the theory of maturation arrest as the mechanism

of tumorigenesis (4). The proliferation of new stage-specific monoclonal antibodies will certainly help to pinpoint the hematologic neoplasms in highly defined stages.

The classification of hematologic neoplasms, therefore, depends on many parameters, including cell morphology, size, stage of maturation, and clinical course. With the recent advances in new technologies, the classification is also based on immunophenotypes, immunogenotypes, and karyotypes. However, the basic requirement for diagnosis is still cytologic recognition of the neoplastic cells. Therefore, it should be useful to be acquainted with the ontogeny of blood cells. This is particularly relevant to biphenotypic lymphoma and/or leukemia and to those tumors derived from a pluripotent stem cell or progenitor cells. Having this knowledge can also help us to understand why, for instance, chronic myelogenous leukemia may have a blast crisis of myeloid, lymphoid, erythroid, or megakaryocytoid origin and why erythroid leukemia has coexistent myeloblasts and trilineage dysplasia.

DEVELOPMENTAL STAGES OF HEMATOPOIETIC CELLS

During fetal life, hematopoiesis initially takes place in the yolk sac and occurs later in the liver and spleen. After birth, the hematopoietic function is taken over by the bone marrow. All the blood cells are derived from a pluripotent stem cell that can differentiate into various lineages, including erythrocytes, megakaryocytes, basophils, eosinophils, neutrophils, monocytes, and lymphocytes (Fig. 5.1). The precursors of these lineages are called colony-forming units. Precursors of T lymphocytes must go to the thymus, where they develop through several stages of thymocytes and finally become mature T lymphocytes. The mature T lymphocytes exit the thymus, enter into the blood and lymph, and reach the peripheral lymphoid tissues. B lymphocytes do not go through the thymus but mature in the bone marrow and enter the lymphoid tissue via blood and lymph. The third lineage of lymphoid cells was initially called null cell, because it lacks surface immunoglobulin and does not form sheep erythrocyte rosettes. It is now recognized that most null cells are large granular lymphocytes that are able

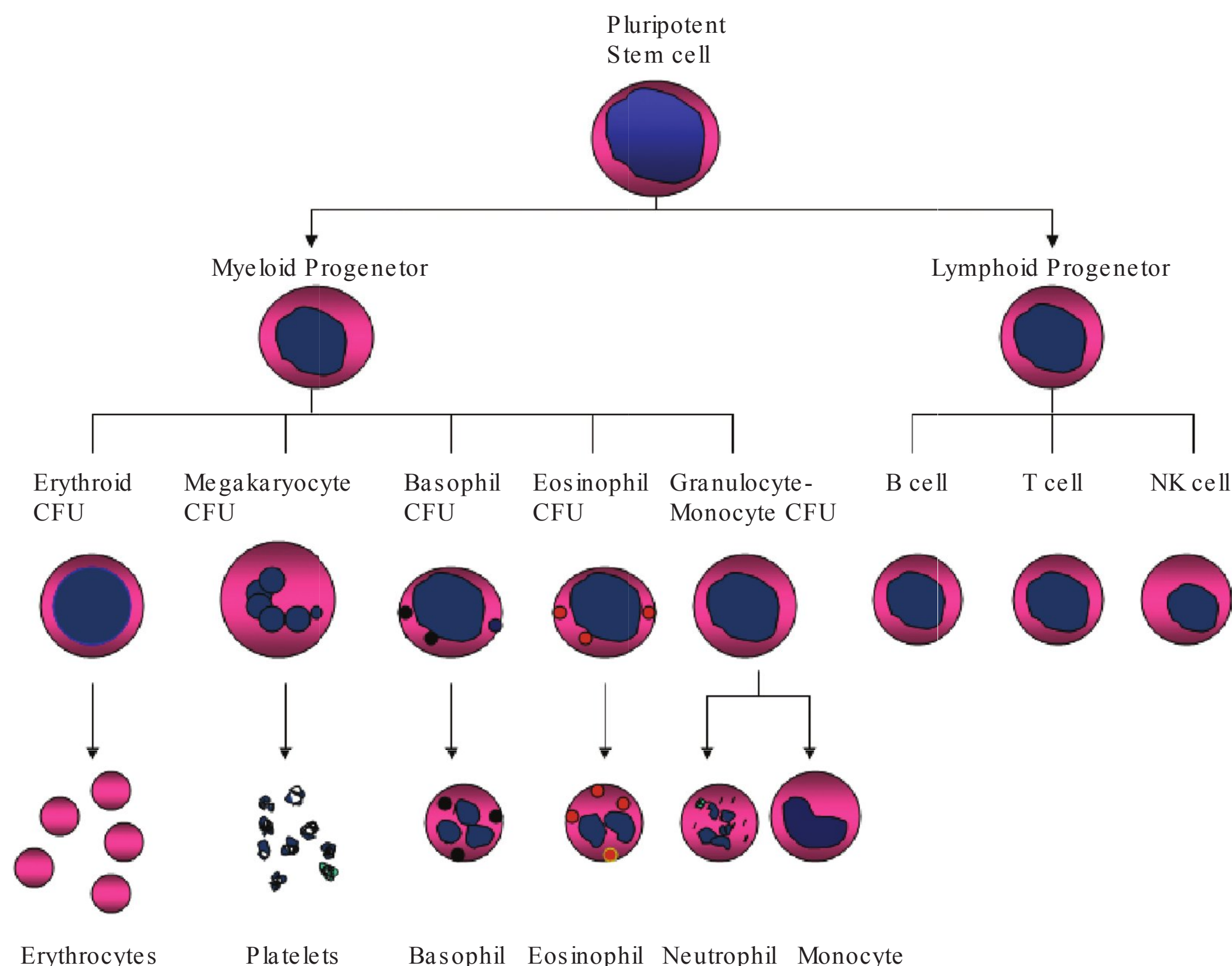


FIGURE 5.1 Development of hematopoietic cells (hematopoietic tree). CFU, colony-forming unit.

to lyse a variety of tumor cells and virus-infected cells and are thus called natural killer (NK) cells (5).

The proliferation and maturation of various precursors are stimulated by cytokines called colony-stimulating factors (CSFs) (5). T lymphocytes produce interleukin-3 (IL-3), which stimulates all precursors, and granulocyte-monocyte CSF (GM-CSF). Macrophages and marrow stromal cells also produce GM-CSF and additional CSFs specific for granulocytes (G-CSF) or monocytes (M-CSF), as well as IL-1 and IL-6. The interleukins can enhance colony formation by hematopoietic precursors in the presence of CSFs. The bone marrow stromal cells can also produce IL-7, which preferentially stimulates the maturation of B lymphocytes.

The development of T lymphocytes starts in the thymus. The earliest stage of thymocyte is called stage I thymocyte or prothymocyte, which represents 13% of the entire thymocyte population (Table 5.1) (6,7). It expresses terminal deoxynucleotidyl transferase (TdT) in the nucleus; CD3 in the cytoplasm; and CD38, CD71, and the earliest T-cell surface marker, CD7, on the surface. Some studies also indicated the presence of surface HLA-DR, CD34, and possibly CD2 at this stage. Stage II thymocytes, also called common thymocytes, include subcapsular and cortical cells and represent approximately 75% of the total thymocyte population. In this stage, CD1, CD2, CD5, and CD7 are expressed. CD4 and CD8 are usually coexpressed or only one of these two is expressed. Stage III thymocytes, also called medullary or mature thymocytes, consist of about 15% of the thymocyte population. The mature thymocytes express surface CD3 and T-cell receptor protein and start to divide

into CD4+, CD8-, and CD4-, CD8+ subgroups. When the mature thymocytes enter into the peripheral circulation, they become postthymic or peripheral T cells, which lose the CD38 and TdT markers.

CD4 T cells are further differentiated into Th1, Th2, Th17, and regulatory T cells, depending on the pattern of signals they received during their initial interaction with antigen (8). A specific subtype of effector T cells is the follicular helper T cell that is located in the germinal center with a characteristic phenotype of CD3+, CD4+, CD10+, BCL-6+.

NK cells are originated from bone marrow-derived CD34+ CD45RA+ hematopoietic progenitor cells (HPCs) (9). HPCs circulate in the blood and extravasate across lymph node high endothelial venules to enter the parafollicular space. In the lymph node or other secondary lymphoid tissue (SLT), progenitor NK cells are activated by dendritic cells to progress through distinct stages of maturation (preNK and immature NK or iNK) to produce CD56^{bright} and CD56^{dim} NK cells. Maturing CD56^{dim} NK cells return to the circulation via the efferent lymph. Some of the CD56^{bright} NK cells remain within the SLT to interact with dendritic cells. Therefore, SLT serves as the maturation site for NK cells, similar to thymus for T cells.

The development of B cells is confined to the bone marrow. In the B-cell progenitor stage, only TdT, HLA-DR, CD34, and cytoplasmic CD79A are expressed (Table 5.2) (6,7,10). The pre-B-cell stage is characterized by the presence of cytoplasmic μ chain without accompanying light chain and the loss of CD34. CD19, CD20, cytoplasmic CD22, and PAX5 first appear at this stage. In the immature B-cell

TABLE 5.1

Immunophenotype of Different Developmental Stages of T Lymphocytes

Stage I Prothymocyte	Stage II Common thymocyte	Stage III Mature thymocyte	Postthymic T Lymphocyte
TdT	TdT	TdT	TCR
cCD3	cCD3	cCD3	CD3
CD7	CD7	CD7	CD7
CD2 (±)	CD2	CD2	CD2
CD34	CD5	CD5	CD5
HLA-DR	CD1	CD4 or CD8	CD4 or CD8
CD38	CD4	CD3	
CD71	CD8	TCR	
	CD38	CD38	

cCD3, cytoplasmic CD3; TCR, T-cell receptor (TCRab 95%, TCRgd 5%); TdT, terminal deoxynucleotidyl transferase.

stage, surface immunoglobulin light chains and CD21 start to appear. CD10 usually disappears at this stage, but it is frequently present on Burkitt lymphoma cells, which are considered immature B cells (11). When B cells become mature, immunoglobulin D (IgD) appears on the surface side-by-side with IgM. The mature B cells are considered resting or “virgin” until activated by their contact with antigens in the germinal center. As heavy-chain switching occurs upon B-cell activation, the activated B cells (germinal B cells) express surface IgM, IgA, or IgG instead of IgM/IgD. CD21 disappears at this stage, but activation antigens, such as CD38 and CD71, and proliferation-associated antigen, Ki-67, are frequently expressed. Activated B cells finally develop into the terminal stage, plasma cell. Plasma cells synthesize cytoplasmic immunoglobulin and express surface CD38, CD79A, CD138, PCA-1, and PC-1. Some

activated B cells may become memory (marginal zone) B cells, which have an immunophenotype similar to that of either a resting B cell (7) or an activated B cell (12).

The circulating lymphocytes may migrate to the lymph nodes, the mucosal follicles, and other extranodal sites depending on their surface-homing receptors for high-endothelial venules in various tissues (13). In the lymph node, lymphocytes travel from one compartment to another, undergoing further morphologic changes.

Intranodal B-Cell Differentiation

The lymph node contains four compartments: (a) the cortex, (b) paracortex, (c) medullary cords, and (d) sinuses. The cortex is composed of lymphoid follicles. A primary follicle contains aggregates of resting or virgin B cells (12,14). After antigenic stimulation, resting B lymphocytes

TABLE 5.2

Immunophenotypes of Different Developmental Stages of B Lymphocytes

Progenitor B cell: TdT, CD34, cCD79A

β

Pre-B cell: TdT, CD19, CD10, CD20, CD79A, cCD22, PAX5, C_m

β

Immature B cell: CD19, CD20, CD79A, CD21, cCD22, PAX5, sIgM

β

Mature B cell: CD19, CD20, CD21, CD22, CD79A, PAX5, sIgM/IgD

β

Activated B cell: CD19, CD20, CD22, CD79A, PAX5, sIgM or IgG

β

Plasma cell: cytoplasmic immunoglobulins, CD38, CD79A, CD138, PCA-1, PC-1

c, cytoplasmic; s, surface; TdT, terminal deoxynucleotidyl transferase; Ig, immunoglobulin.

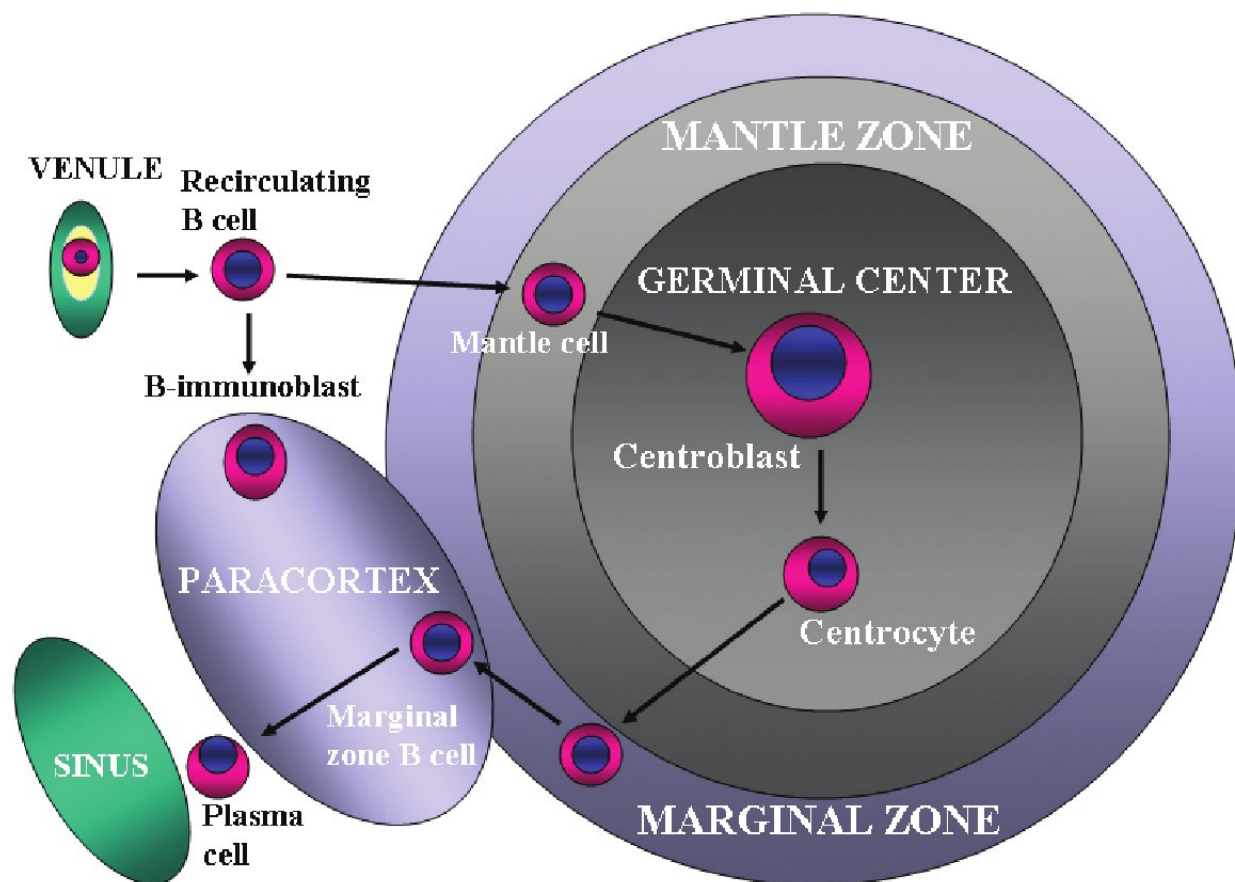


FIGURE 5.2 Intranodal B-cell differentiation (maturation). Recirculating B cells migrate through the high-endothelial venule in the hilum of lymph node to the mantle zone, germinal center, marginal zone, and finally, sinus.

become activated, leading to proliferation and blastic transformation. A germinal center is formed and is surrounded by a mantle zone, which is made up of the same resting B cells as those in the primary follicles. The follicle with a germinal center is called a secondary follicle. In the germinal center, B cells are activated by antigens and start to proliferate and undergo somatic mutation. Generation of memory cells and plasma cells and heavy-chain class switch also take place in the germinal center (15). In the germinal center, high-affinity antigen-specific B cells are positively selected as the result of their interaction with antigen–antibody complexes on the surface of follicular dendritic cells. B cells that are not positively selected undergo apoptosis and are phagocytized by tangible-body macrophages (15).

On the basis of current studies, the sequence of normal B-cell differentiation within the lymph node is suggested as follows (Fig. 5.2) (10,12,16). Immature B cells from the bone marrow first migrate to the mantle zone and become the mantle cell. At the same time, immature B cells also migrate to the interfollicular area and become extrafollicular B blasts, which may transform into short-lived plasma cells or enter into the germinal center and become centroblasts. The centroblasts finally evolve through the stages of small noncleaved cell, large noncleaved cell, and small cleaved cell (follicular center cells) and become centrocytes.

Some activated B cells transform into memory B cells and migrate to the marginal zone to become marginal zone cells (17). Some marginal zone cells have ovoid nuclei and relatively abundant clear cytoplasm resembling monocytes and are thus called monocytoid B cells. Centrocytes and marginal zone cells may transform into effector cells and become long-lived plasma cells (10). The plasma cell is the terminal stage of the B cell, which moves to the medullary cord and finally migrates back to the bone marrow.

Pregerminal Center, Germinal Center, and Postgerminal Center Lymphomas

Lymphoma can develop at each stage of intranodal differentiation. Accordingly, there are mantle cell lymphoma, follicular lymphoma, nodal marginal zone lymphoma, lymphoplasmacytic lymphoma, and plasma cell myeloma. The origin of a lymphoma can be determined by the status of V_H gene mutation. Lymphomas that show no V_H gene mutation represent a tumor from the pregerminal center. Lymphomas that express V_H gene mutation and intraclonal diversity are derived from the germinal center, whereas those that have V_H gene mutation but not intraclonal diversity are originated from postgerminal center B cells.

Pregerminal center lymphoma is represented by mantle cell lymphoma (10). Germinal center lymphoma includes follicular lymphoma, Burkitt lymphoma, and a subset of diffuse large B-cell lymphoma. Hodgkin lymphoma is also in this category. Postgerminal center lymphoma includes B-CLL/SLL, nodal marginal zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma, splenic marginal zone lymphoma, plasma cell myeloma, lymphoplasmacytic lymphoma, and a subset of diffuse large B-cell lymphoma.

CLASSIFICATION BASED ON CLINICAL PRESENTATION

Lymphomas of different origins also may show different clinical presentations. In the World Health Organization (WHO) classification, the mature B-cell neoplasms are divided into three groups, according to their major clinical presentations (16).

1. Predominantly disseminated lymphoma and/or leukemia: This group of tumors usually involves the bone marrow with or without peripheral blood and solid tissues, such as lymph nodes and spleen, involvement. It includes CLL, lymphoplasmacytic lymphoma, hairy cell leukemia, splenic marginal zone lymphoma, and plasma cell myeloma.
2. Primary extranodal lymphomas: This group of tumors virtually always presents in extranodal sites. It is represented by extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT).
3. Predominantly nodal lymphomas: This group of tumors involves the lymph nodes initially but frequently spreads to the bone marrow, liver, spleen, and peripheral blood. It includes follicular lymphoma, mantle cell lymphoma, and nodal marginal zone B-cell lymphoma.
4. Two aggressive B-cell lymphomas may present with either nodal or extranodal disease. These are diffuse large B-cell lymphoma and its variants and Burkitt lymphoma.

CLASSIFICATION OF ACUTE LEUKEMIAS

The French-American-British (FAB) classification has been used as the basis for the classification of acute leukemia for many years (18,19). However, the WHO

TABLE 5.3

2008 WHO Classification for Precursor Lymphoid Neoplasms

B lymphoblastic leukemia/lymphoma, not otherwise specified**B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities**

B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2); BCR-ABL1

B lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged

B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22); TEL-AML 1 (ETV6-RUNX1)

B lymphoblastic leukemia/lymphoma with hyperdiploidy

B lymphoblastic leukemia/lymphoma with hypodiploidy (hypodiploid ALL)

B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); IL3-IGH

B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); E2A-PBX1 (TCF3-PBX1)

T lymphoblastic leukemia/lymphoma

classification has made many changes to the FAB classification. The FAB classification divides ALL into L1, L2, and L3, but the WHO classification considers that the division of L1 and L2 does not serve any clinical purpose and merges them into precursor B-cell and precursor T-cell ALLs (10,20). Burkitt leukemia is excluded from L3 in the 2008 WHO classification. B lymphoblastic leukemia/lymphoma is further divided into B lymphoblastic leukemia/lymphoma, not otherwise specified and B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities (Table 5.3) (10).

In acute myeloid leukemia (AML), the original FAB categories, M0, M1, M2, M4, M5, M6, M7, are now classified in the category of AML not otherwise categorized (Table 5.4). This category also includes acute basophilic leukemia, and acute panmyelosis with myelofibrosis. Besides this category, the major addition is the AML with recurrent cytogenetic abnormalities, which includes nine well-defined clinical entities. Myeloid sarcoma, myeloid proliferations related to Down syndrome, and blastic plasmacytoid dendritic cell neoplasm are also included as new entities of AML (10).

CLASSIFICATION OF CHRONIC LEUKEMIA

In the WHO classification, several chronic myeloid leukemias are in the category of myeloproliferative diseases: chronic myelogenous leukemia, chronic neutrophilic leukemia, and chronic eosinophilic leukemia or hypereosinophilic syndrome (17). The chronic lymphoid leukemias, including CLL, Sézary syndrome, large granular lymphocytic leukemia, prolymphocytic leukemia, and adult T-cell leukemia, are in the category of lymphoid neoplasms in the WHO classification (10,20–22).

CLASSIFICATION OF LYMPHOMA

Modern classification of non-Hodgkin lymphoma started with Rappaport (23), whose classification was based on the histologic pattern (nodular or diffuse), cytology (lymphocyte or histiocyte), and cell differentiation (well differentiated or poorly differentiated). The classification of Lukes and Collins (24) combined immunologic subtypes with morphology and proposed that immunologic phenotypes could be determined by morphologic features. The Kiel (22) classification proposed by Lennert and associates is mainly morphologic with more cell types included. The updated Kiel classification further divides lymphoma into T-cell and B-cell categories as well as low-grade and high-grade groups (Table 5.5) (25). The lesser known schemes include Dorfman, the British National Lymphoma Investigation, and the “old” WHO (U.N. World Health Organization) classifications. These six different schemes unavoidably caused some confusion among pathologists; thus, the National Cancer Institute in the United States organized a team of experts to evaluate the available classifications and establish a “compromised” new scheme. As a result, a working formulation of non-Hodgkin lymphomas for clinical use was proposed (26). The Working Formulation is relatively simple and yet incorporates all the major components from other schemes (Table 5.6). Its major advantage is dividing the lymphomas into three prognostic groups that make the Working Formulation clinically relevant. It was promptly accepted and had been widely used, especially in North America.

The Working Formulation, however, does not identify individual disease entities and does not include many new entities, especially in the T-cell lymphoma category, that have appeared in recent years. In addition, the new treatments used currently have changed the outlook of many diseases; thus, the prognostic grouping may no longer be

TABLE 5.4

2008 WHO Classification of AML

AML with recurrent genetic abnormalities

AML with t(8;21)(q22;q22), RUNX1-RUNX1T1

AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22), CBFb-MYH11

Acute promyelocytic leukemia with t(15;17)(q22;q12), PML-RARa (AML-M3)

AML with t(9;11)(p22;q23); MLLT3-MLL

AML with t(6;9)(p23;q34); DEK-NUP214

AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EV11

AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1

AML with mutated NPM1

AML with mutated CEBPA

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML, not otherwise categorized

AML, with minimal differentiation (AML-M0)

AML without maturation (AML-M1)

AML with maturation (AML-M2)

Acute myelomonocytic leukemia (AML-M4)

Acute monoblastic and monocytic leukemia (AML-M5)

Acute erythroid leukemia (AML-M6)

Acute megakaryoblastic leukemia (AML-M7)

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

FAB classification in parenthesis.

valid for some of the lymphomas. Therefore, some American hematologists and oncologists believed that the Working Formulation had outlived its usefulness. Because of this situation, a revised European–American Classification of Lymphoid Neoplasms (REAL classification) was proposed (Table 5.7) (27). This new scheme encompasses many new entities, covers both Hodgkin lymphoma and non-Hodgkin lymphoma, and incorporates immunophenotypes and cytogenetics as integral parts of the diagnosis. Therefore, this classification had become very popular in large medical centers around the world, where immunophenotyping and molecular genetic analysis could be performed.

The REAL classification, however, contains a number of provisional entities that required additional studies for confirmation or elimination in future schemes. The 2001 WHO classification fulfills this function by verifying these provisional entities (Table 5.8) (20–22). For instance, the hepatosplenic gd T-cell lymphoma, nodal

marginal zone B-cell lymphoma, and subcutaneous panniculitis-like T-cell lymphoma are retained, whereas Hodgkin-like anaplastic large cell lymphoma, Burkitt-like lymphoma, and T-cell CLL are eliminated because they are resolvable into other definitive diagnoses. Primary effusion lymphoma and intravascular large B-cell lymphoma are added as subtypes of diffuse large B-cell lymphoma. In addition, follicular center cell lymphoma has been changed into follicular lymphoma, angiocentric lymphoma becomes nasal T/NK-cell lymphoma, and lymphoplasmacytoid lymphoma reverts to lymphoplasmacytic lymphoma. However, the basic principle of the REAL classification still remains in the WHO scheme, and this new classification is sometimes referred to as the updated REAL classification (18).

The 2008 WHO classification greatly expands the scope of lymphoid neoplasms and further defines new entities on the basis of karyotype, age of patients, tumor locations,

TABLE 5.5
Updated Kiel Classification of Non-Hodgkin Lymphoma

B cell	T cell
Low-grade malignant lymphoma	Low-grade malignant lymphoma
Lymphocytic	Lymphocytic
Chronic lymphocytic leukemia	Chronic lymphocytic leukemia
Prolymphocytic leukemia	Prolymphocytic leukemia
Hairy cell leukemia	Small cell, cerebriform
Lymphoplasmacytic/cytoid (immunocytoma)	Mycosis fungoides/Sézary syndrome
Plasmacytic	Lymphoepithelioid (Lennert lymphoma)
Centroblastic/centrocytic (follicular ± diffuse; diffuse)	Angioimmunoblastic (AILD)
Centrocytic (mantle cell)	T-zone lymphoma
Monocytoid, including marginal zone cell	Pleomorphic, small cell (HTLV-1±)
High-grade malignant lymphoma	High-grade malignant lymphoma
Centroblastic	Pleomorphic, medium sized and large cell (HTLV-1±)
Immunoblastic	Immunoblastic (HTLV-1±)
Burkitt lymphoma	Large-cell anaplastic (Ki-1+)
Large-cell anaplastic (Ki-1+)	Lymphoblastic
Lymphoblastic	

AILD, angioimmunoblastic lymphoproliferative disorder; HTLV-1, human T-cell lymphotropic virus type 1.

and infectious agent association. It also recognizes unclassifiable lymphomas and mixed phenotype leukemias (Table 5.9) (10).

MULTILINEAGE PHENOTYPE

A complicated problem accompanying multimarker analysis is the discovery of multilineage phenotypes, which are sometimes referred to as lineage promiscuity (if it is due to the differentiation of a progenitor cell) or lineage infidelity (if due to aberrant gene regulation) (28). Many terms in this area, such as bilineal, biclonal, biphenotypic, and hybrid form, are poorly defined, and their distinction from each other has not been delineated. Therefore, some cases may not be classifiable, and there is an obvious need to standardize these nomenclatures.

In the 2001 WHO scheme, this group of leukemias is classified under the heading of acute leukemias of ambiguous lineage. Bilineal acute leukemia is defined as leukemia with a dual population of blasts with each population expressing markers of distinct lineage, that

is, myeloid and lymphoid. Biphenotypic acute leukemia is defined as leukemia with blasts coexpressing myeloid- and lymphoid-specific antigens (29). However, the coexpression of only one or two cross-lineage antigens is not a sufficient criterion to diagnose biphenotypic leukemia. For instance, the expression of CD13 and CD33 in a case of ALL should be called myeloid–antigen–positive ALL. The European Group for the Immunologic Classification of Leukemia has proposed a scoring system for the relative specificity of various lineage-associated markers (see Case 16, Table 6.16.1) (30).

The 2008 WHO classification further divides acute leukemias of ambiguous lineage into acute undifferentiated leukemia; mixed phenotype acute leukemia with t(9;22)(q34;q11.2); BCR-ABL1; mixed phenotype acute leukemia with t(v;11q23); MLL rearranged; mixed phenotype acute leukemia, B/myeloid, NOS; mixed phenotype acute leukemia, T/myeloid, NOS; Mixed phenotype acute leukemia, NOS—rare types; other ambiguous lineage leukemias; natural killer–cell lymphoblastic leukemia/lymphoma (10).

(text continues on Page 50)



TABLE 5.6
Working Formulation of Non-Hodgkin Lymphoma for Clinical Usage
Low grade
A. Malignant lymphoma, small lymphocytic
Consistent with CLL
Plasmacytoid
B. Malignant lymphoma, follicular, predominantly small cleaved cell
Diffuse area
Sclerosis
C. Malignant lymphoma, follicular, mixed
Small cleaved and large cell
Diffuse areas
Sclerosis
Intermediate grade
D. Malignant lymphoma, follicular, predominantly large cell
Diffuse areas
Sclerosis
E. Malignant lymphoma, diffuse, small cleaved cell
Sclerosis
F. Malignant lymphoma, diffuse, mixed
Small/large cell
Sclerosis
G. Malignant lymphoma, diffuse, large cell
Cleaved cell
Noncleaved cell
Sclerosis
High grade
H. Malignant lymphoma, large cell, immunoblastic
Plasmacytoid
Cleaved cell
Polymorphous
Epithelial cell component
I. Malignant lymphoma, lymphoblastic
Convolut cell
Nonconvolut cell
J. Malignant lymphoma, small noncleaved cell
Burkitt
Follicular areas
Miscellaneous
Composite
Mycosis fungoides
Histiocytic
Extramedullary plasmacytoma
Unclassifiable
Other

CLL, chronic lymphocytic leukemia.

TABLE 5.7

Revised European–American Classification of Lymphoid Neoplasms

B-cell neoplasms

I. Precursor B-cell neoplasm: Precursor B-lymphoblastic leukemia/lymphoma

II. Peripheral B-cell neoplasms

- 1. B-cell CLL/prolymphocytic leukemia/SLL
- 2. Lymphoplasmacytoid lymphoma/immunocytoma
- 3. Mantle cell lymphoma
- 4. Follicle center lymphoma, follicular

Provisional cytologic grades: I (small cell), II (mixed small/large cell), III (large cell)

Provisional subtype: Diffuse, predominantly small-cell type

- 5. Marginal zone B-cell lymphoma
Extranodal (MALT type ± monocytoid B cells)
- 6. Provisional subentity: Splenic marginal zone lymphoma (± monocytoid B cells)
- 7. Hairy cell leukemia
- 8. Plasmacytoma/plasma cell myeloma
- 9. Diffuse large B-cell lymphoma*
- 10. Burkitt lymphoma
- 11. Provisional entity: High-grade B-cell lymphoma, Burkitt-like*

T-cell and putative NK-cell neoplasms

I. Precursor T-cell neoplasm: Precursor T-lymphoblastic lymphoma/leukemia

II. Peripheral T-cell and NK-cell neoplasms

- 1. T-cell CLL/prolymphocytic leukemia
- 2. Large granular lymphocyte leukemia (LGL)
T-cell type
NK-cell type

- 3. Mycosis fungoides/Sézary syndrome
- 4. Peripheral T-cell lymphoma, unspecified*

Provisional cytologic categories, medium-size cell, mixed medium/large cell, large cell, lymphoepithelioid cell

Provisional subtype: Hepatosplenic gd T-cell lymphoma

Provisional subtype: Subcutaneous panniculitic T-cell lymphoma

- 5. Angioimmunoblastic T-cell lymphoma (AILD)
- 6. Angiocentric lymphoma
- 7. Intestinal T-cell lymphoma (± enteropathy associated)
- 8. Adult T-cell lymphoma/leukemia (ATL/L)
- 9. Anaplastic large cell lymphoma (ALCL), CD30+, T– and null-cell types
- 10. Provisional entity: Anaplastic large cell lymphoma, Hodgkin-like

Hodgkin lymphoma

- I. Lymphocyte predominance
- II. Nodular sclerosis
- III. Mixed cellularity
- IV. Lymphocyte depletion
- V. Provisional entity: Lymphocyte-rich classic Hodgkin lymphoma

*These categories are likely to include more than one disease entity.
CLL, chronic lymphocytic leukemia; MALT, mucosa-associated lymphoid tissue; NK, natural killer; SLL, small lymphocytic lymphoma.

TABLE 5.8

2001 WHO Classification of Lymphoid Neoplasms

B-cell neoplasms Precursor B-cell neoplasms Precursor B-lymphoblastic leukemia/lymphoma (precursor B-cell ALL) Mature (peripheral) B-cell neoplasms B-cell CLL/SLL B-cell prolymphocytic leukemia Lymphoplasmacytic lymphoma Splenic marginal zone lymphoma Hairy cell leukemia Plasma cell myeloma/plasmacytoma Extranodal marginal zone B-cell lymphoma or MALT type Nodal marginal zone B-cell lymphoma Follicular lymphoma Mantle cell lymphoma Diffuse large B-cell lymphoma Mediastinal large B-cell lymphoma Primary effusion lymphoma Intravascular large B-cell lymphoma Burkitt lymphoma/Burkitt cell leukemia
T- and NK-cell neoplasms Precursor T-cell neoplasm Precursor T-lymphoblastic lymphoma/leukemia (precursor T-cell ALL) Mature (peripheral) T-cell neoplasms T-cell prolymphocytic leukemia T-cell large granular lymphocytic leukemia Aggressive NK-cell leukemia Adult T-cell lymphoma/leukemia (HTLV-1+) Extranodal NK/T-cell lymphoma, nasal type Enteropathy-type T-cell lymphoma Hepatosplenic T-cell lymphoma Subcutaneous panniculitis-like T-cell lymphoma Mycosis fungoides/Sézary syndrome Anaplastic large cell lymphoma, T/null cell, primary systemic type Anaplastic large cell lymphoma, T/null cell, primary cutaneous type Peripheral T-cell lymphoma, unspecified Angioimmunoblastic T-cell lymphoma
Hodgkin lymphoma (Hodgkin disease) Nodular lymphocyte predominance Hodgkin lymphoma Classic Hodgkin lymphoma Nodular sclerosis Hodgkin lymphoma Lymphocyte-rich classical Hodgkin lymphoma Mixed-cellularity Hodgkin lymphoma Lymphocyte depletion Hodgkin lymphoma

ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; HTLV-1, human T-cell lymphotropic virus type 1; MALT, mucosa-associated lymphoid tissue; SLL, small lymphocytic lymphoma.

TABLE 5.9

2008 WHO Classification of Lymphoid Neoplasms

B-cell neoplasms

Precursor B-cell neoplasms

B lymphoblastic leukemia/lymphoma, NOS

B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities*

Mature (peripheral) B-cell neoplasms

B-cell CLL/SLL

B-cell prolymphocytic leukemia

Splenic B-cell marginal zone lymphoma

Hairy cell leukemia

Splenic B-cell lymphoma/leukemia, unclassifiable

Splenic diffuse red pulp small B-cell lymphoma

Hairy cell leukemia variant

Lymphoplasmacytic lymphoma

Waldenström macroglobulinemia

Heavy chain diseases

Alpha heavy chain disease

Gamma heavy chain disease

Mu heavy chain disease

Plasma cell myeloma

Solitary plasmacytoma of bone

Extraosseous plasmacytoma

Extranodal marginal zone lymphoma of MALT type

Nodal marginal zone lymphoma

Pediatric nodal marginal zone lymphoma

Follicular lymphoma

Pediatric follicular lymphoma

Primary cutaneous follicle center lymphoma

Mantle cell lymphoma

Diffuse large B-cell lymphoma (DLBCL), NOS

T-cell/histiocyte rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV positive DLBCL of the elderly

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

Primary mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

ALK positive large B-cell lymphoma

Plasmablastic lymphoma

Large B-cell lymphoma arising in HHV8-associated multicentric Castleman disease

Primary effusion lymphoma

Burkitt lymphoma

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma

(continued)

TABLE 5.9 (continued)

2008 WHO Classification of Lymphoid Neoplasms

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

T- and NK-cell neoplasms

Precursor T-cell neoplasms

T lymphoblastic leukemia/lymphoma

Mature T-cell and NK-cell neoplasms

T-cell prolymphocytic leukemia

T-cell large granular lymphocytic leukemia

Chronic lymphoproliferative disorder of NK cells

Aggressive NK-cell leukemia

Systemic EBV positive T-cell lymphoproliferative disease of childhood

Hydroa vacciniforme-like lymphoma

Adult T-cell leukemia/lymphoma

Extranodal NK/T-cell lymphoma, nasal type

Enteropathy-associated T-cell lymphoma

Hepatosplenic T-cell lymphoma

Subcutaneous panniculitis-like T-cell lymphoma

Mycosis fungoides

Sézary syndrome

Primary cutaneous CD30 positive T-cell lymphoproliferative disorders

Lymphomatoid papulosis

Primary cutaneous anaplastic large cell lymphoma

Primary cutaneous gamma-delta T-cell lymphoma

Primary cutaneous CD8 positive aggressive epidermotropic cytotoxic T-cell lymphoma

Primary cutaneous CD4 positive small/ medium T-cell lymphoma

Peripheral T-cell lymphoma, NOS

Angioimmunoblastic T-cell lymphoma

Anaplastic large cell lymphoma, ALK positive

Anaplastic large cell lymphoma, ALK negative

Hodgkin lymphoma

Nodular lymphocyte predominant Hodgkin lymphoma

Classical Hodgkin lymphoma

Nodular sclerosis classical Hodgkin lymphoma

Lymphocyte-rich classical Hodgkin lymphoma

Mixed cellularity classical Hodgkin lymphoma

Lymphocyte-depleted classical Hodgkin lymphoma

CLL, chronic lymphocytic leukemia; DLBCL, diffuse large B-cell lymphoma; MALT, mucosa-associated lymphoid tissue; NK, natural killer; SLL, small lymphocytic lymphoma; provisional entities are in italic type.

*see Table 5.3

REFERENCES

1. Freedman AS, Nadler LM. Immunologic markers in non-Hodgkin's lymphoma. *Hematol Oncol Clin North Am.* 1991;5:871–889.
2. Ghobrial IM, Bone ND, Stenson MJ, et al. Expression of the chemokine receptors CXCR4 and CCR7 and disease progression in B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma. *Mayo Clin Proc.* 2004;79:318–325.
3. Calyberger C, Wright A, Medeiros LJ, et al. Absence of cell surface LFA-1 as a mechanism of escape from immunosurveillance. *Lancet.* 1987;2:533–536.
4. Salmon SE. B-cell neoplasia in man. *Lancet.* 1974;2:1230–1233.
5. Abbas AK, Lichtman AH, Pillai S. *Cellular and Molecular Immunology.* 7th ed. Philadelphia, PA: W. B. Saunders; 2011:15–35.
6. Knowles DM. Immunophenotypic markers useful in the diagnosis and classification of hematopoietic neoplasms. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:93–226.
7. Stetler-Stevenson M, Medeiros LJ, Jaffe ES. Immunophenotypic methods and findings in the diagnosis of lymphoproliferative diseases. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs.* 2nd ed. Philadelphia, PA: W. B. Saunders; 1995:22–57.
8. Zhu J, Paul WE. CD4 T cells:fates, functions and faults. *Blood.* 2008;112:461–469.
9. Caligiuri MA. Human natural killer cells. *Blood.* 2008;112:461–469.
10. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2008.
11. Garcia CR, Weiss LM, Warnke RA. Small noncleaved cell lymphoma: an immunophenotypic study of 18 cases and comparison with large cell lymphoma. *Hum Pathol.* 1986;17:454–461.
12. Weisenberger DD, Chan WC. Lymphoma of follicles, mantle cell and follicle center cell lymphoma. *Am J Clin Pathol.* 1993;99:409–420.
13. Butcher E. Cellular and molecular mechanisms that direct leukocyte traffic. *Am J Pathol.* 1990;136:3–12.
14. Gloghini A, Carbone A. The nonlymphoid microenvironment of reactive follicles and lymphomas of follicular origin as defined by immunohistology on paraffin embedded tissues. *Hum Pathol.* 1993;24:67–76.
15. Delves PJ, Poitt IM. The immune system: second of two parts. *N Engl J Med.* 2000;343:108–117.
16. Harris NL. Mature B-cell neoplasms: introduction. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:121–126.
17. Harris NL. Low-grade B-cell lymphoma of mucosa-associated lymphoid tissue and monocytoid B-cell lymphoma. *Arch Pathol Lab Med.* 1993;117:771–775.
18. Bennett JM, Catovsky D, Daneil MT, et al. French-American-British (FAB) Cooperative Group. Proposals for the classification of acute leukemias. *Br J Haematol.* 1976;33:451–458.
19. Bennett JM, Catovsky DD, Flandrin G, et al. Proposed revised criteria for the classification of acute myeloid leukemia. *Ann Intern Med.* 1985;103:626–629.
20. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee Meeting, Airlie House, Virginia, November 1997. *Mod Pathol.* 2000;13:193–207.
21. Harris NL, Jaffe ES, Diebold J, et al. Lymphoma classification—from controversy to consensus: the R.E.A.L. and WHO classification of lymphoid neoplasms. *Ann Oncol.* 2000;11(suppl 1):S3–S10.
22. Isaacson PG. The current status of lymphoma classification. *Br J Haematol.* 2000;109:258–266.
23. Rappaport H. Tumors of the hematopoietic system. In: *Atlas of Tumor Pathology.* Washington, DC: Armed Forces of Institute of Pathology; 1986.
24. Lukes RJ, Collins RD. Immunologic characterization of human malignant lymphomas. *Cancer.* 1974;34:1488–1503.
25. Stansfield AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas. *Lancet.* 1988;1:292–293.
26. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas: summary and description of a working formulation for clinical usage. The Non-Hodgkin's Lymphoma Pathologic Classification Project. *Cancer.* 1982;49:2112–2135.
27. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood.* 1994;84:1362–1392.
28. Cross AH, Goorha RM, Nuss R, et al. Acute myeloid leukemia with T-lymphoid features: a distinct biologic and clinical entity. *Blood.* 1988;72:579–587.
29. Bruning RD, Matutes E, Borowitz M, et al. Acute leukemias of ambiguous lineage. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:106–108.
30. The value of c-kit in the diagnosis of biphenotypic acute leukemia. EGIL (European Group for the Immunological Classification of Leukaemias). *Leukemia.* 1998;12:2038.

Clinical Application

6

The clinical application of immunohistochemistry is discussed in Chapter 3 and that of molecular genetics in Chapter 4; this chapter is devoted mainly to flow cytometric applications. By using a panel of appropriately selected monoclonal antibodies, flow cytometric analysis may determine the cell lineage (T cell, B cell, natural killer [NK] cell, or myelomonocytic cell), developmental stage (mature vs. immature, thymic vs. postthymic), and clonality (monoclonal vs. polyclonal) of a given cell population. It may also determine the heterogeneous and aberrant features and the percentage of the tumor cells (1). The analysis of these parameters by immunologic means is called immunophenotyping.

Immunophenotyping serves many different functions. When the features of cytology and histopathology are not diagnostic, immunophenotyping helps distinguish a benign lesion from a malignant one, thus achieving a definitive diagnosis. Even when diagnosis is not a major problem, immunophenotyping is still needed and plays an essential role in differential diagnosis, subclassification, and prediction of prognosis. These functions are well exemplified in the area of low-grade B-cell lymphomas, such as small lymphocytic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, hairy cell leukemia, and various types of marginal zone B-cell lymphoma, to name just a few. The continuing discovery of new monoclonal antibodies enables better refinement for the diagnosis and classification of these diseases. For instance, the availability of CD23 antibody facilitates the distinction between small lymphocytic lymphoma and mantle cell lymphoma. The prognosis for a certain lymphoma/leukemia can be evaluated by certain markers, such as ZAP70 in chronic lymphocytic leukemia. A poor prognosis may also be expected when high percentages of activation antigens (e.g., CD25, CD38, CD71, and HLA-DR) are present.

CRITERIA FOR FLOW CYTOMETRIC DIAGNOSIS OF HEMATOLOGIC NEOPLASMS

The major drawback of flow cytometry (FC) for the diagnosis of hematologic neoplasms is its inability to correlate the

cell morphology with the surface, cytoplasmic, or nuclear markers, because FC does not allow pathologists or other scientists to have a direct view of the cells examined. Therefore, a set of criteria is established to distinguish hematologic neoplasms from normal leukocytes (Table 6.1).

Immunoglobulin Light-Chain Restriction

The surface immunoglobulin (Ig) light-chain ratio is the most commonly used diagnostic criterion, because it defines the B-cell lineage and clonality at the same time. Its importance is based on the fact that 80% to 90% of lymphomas are of B-cell origin. When one light chain is dominant over the other, it is referred to as light-chain restriction and is indicative of monoclonality. The normal k/l ratio is about 2:1. The definition of monoclonality on the basis of this ratio varies from different studies. Taylor (2) defined a monoclonal pattern as a k/l ratio $\geq 3:1$ or a l/k ratio $\geq 2:1$. Samoszuk et al. (3) defined monoclonality as a k/l ratio of 5.5:1 and a l/k ratio of 1.7:1. By using a higher cutoff point in the k/l ratio, the specificity is increased, whereas the sensitivity is decreased (false-negative rate was 27%). This false-negative rate is probably too high to be acceptable by clinical laboratories. Our experience is that if the B-cell population is <20% of the total population or if the minor light-chain component (either k or l) is >10% (e.g., k 45% and l 15%), the value of the k/l ratio is not reliable.

Taylor (2) also defined monoclonality as the ratio of the predominant heavy chain to the sum of other heavy chains $\geq 3:1$. The argument against the use of heavy chains as markers for clonality is that heavy-chain switch may take place after gene rearrangement, and thus no predominant heavy chain will be detected in that situation. In addition, for cost effectiveness, most laboratories have discontinued the use of three or five heavy-chain antibodies. However, the demonstration of heavy-chain switch is sometimes associated with lymphoma transformation, such as in Richter syndrome (4). Furthermore, the data obtained from heavy-chain analysis can be used to double-check the light-chain results and is useful in borderline cases (5). Nevertheless, for cost containment, most clinical laboratories have discontinued the use of heavy-chain antibodies.

TABLE 6.1

Criteria for Diagnosis of Hematologic Neoplasms by FC

1. Ig light-chain restriction.
2. Loss of surface Ig in a B-cell population.
3. Coexistence of two different cell-lineage markers on the same cell population.
4. Expression of immature cell markers in a large number of cells.
5. Selective loss of one or more cell lineage antigens.
6. Analysis of T-cell receptor-V β repertoire.

For surface Ig studies, polyclonal antibodies should be used because monoclonal antibodies react only to a single antigenic epitope of a particular Ig and do not react to some subclasses of Ig (such as IgG₃ or IgG₄) that may be present exclusively on some tumor cells.

Loss of Surface Immunoglobulin in a B-cell Population

In about 10% to 20% of lymphomas, B-cell antigens are demonstrated on tumor cells that show no surface Ig by immunohistochemical study or a low percentage of Ig-positive cells by flow cytometric analysis (5,6). Because surface Ig is the antigen receptor on normal B cells, the lack of it is found only on neoplastic cells. The common examples that express the surface Ig-negative, B-cell antigen-positive immunophenotype is the primary mediastinal B-cell lymphoma and acute lymphoblastic leukemia of B-cell origin (L1 and L2).

Coexistence of Two Different Cell Lineage Markers on the Same Cell Population

Dual-cell lineage markers have become the hallmark of several lymphoid tumors. For instance, chronic lymphocytic leukemia, small lymphocytic lymphoma, and mantle cell lymphoma carry a B-cell marker (either CD19 or CD20) and a T-cell marker (CD5), whereas hairy cell leukemia bears a B-cell marker (CD22) and a monocyte marker (CD11c). In acute myeloid leukemia, more and more lymphoid markers are being found on the leukemic cells. These double-labeled leukemic cells previously were considered to be bilineal. However, when a single lymphoid marker (e.g., CD7) is coexistent with myeloid markers, it is now considered to be an aberrant phenotype, which is consistent with leukemia rather than a normal myeloid population.

Expression of Immature Cell Markers in a Large Number of Cells

The demonstration of terminal deoxynucleotidyl transferase (TdT) and CD10 (CALL) on tumor cells of lymphoblastic lymphoma and/or acute lymphoblastic leukemia and CD34 (hematopoietic progenitor cell antigen) and CD117 (c-kit or stem cell factor receptor) on cells of acute myeloid leukemia are good examples. The only exception is the presence of hematogones in pediatric bone marrow or in patients

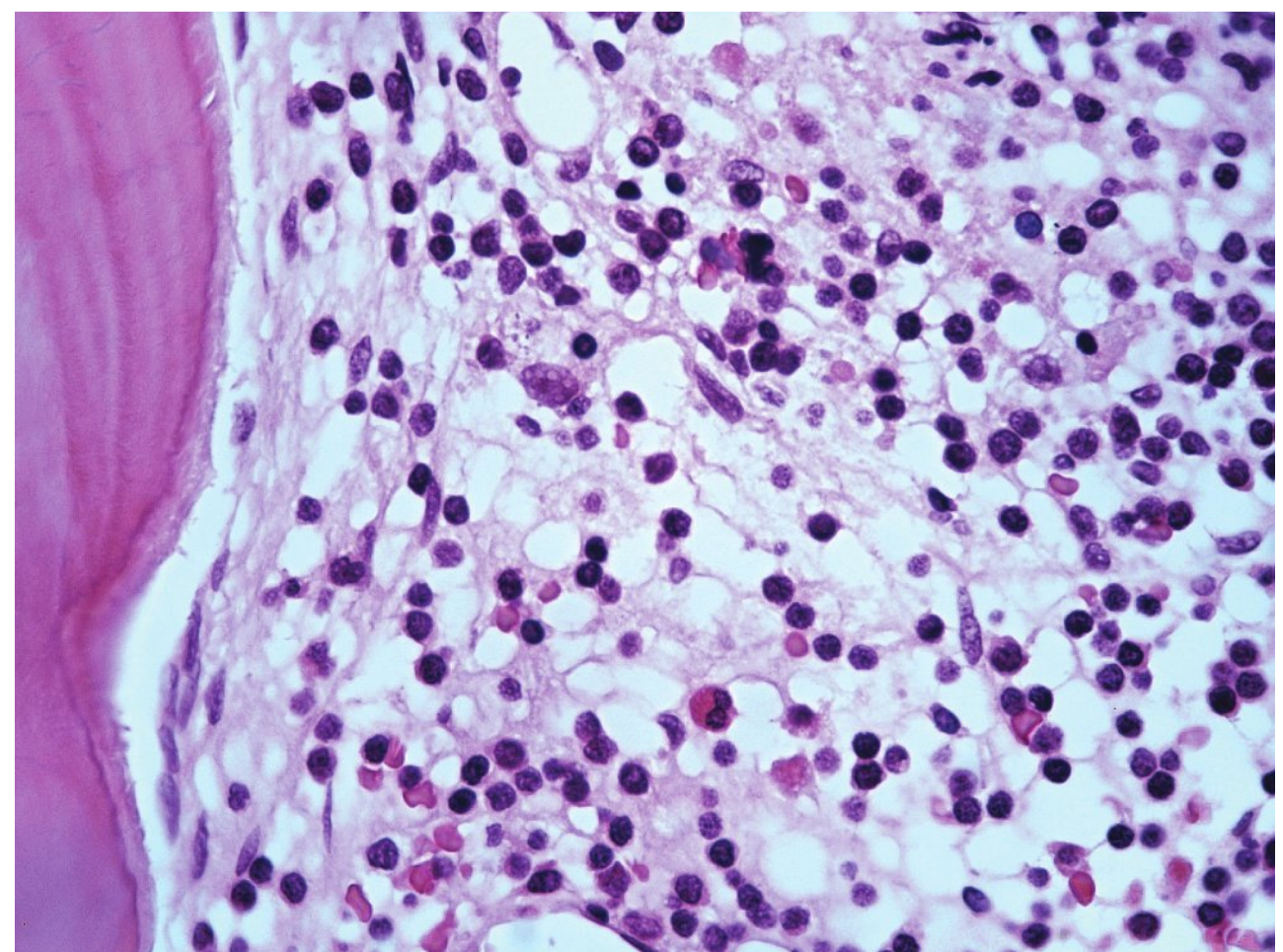


FIGURE 6.1 Bone marrow biopsy from a patient with acute myeloid leukemia after chemotherapy. Note a homogeneous population of small lymphocytes representing hematogones, as proved by FC. Hematoxylin and eosin, 60 $\times$ magnification.

after bone marrow transplantation or chemotherapy (Fig. 6.1). Hematogones are precursors of lymphocytes, which may carry the immature markers, such as CD10, TdT, or CD34, but morphologically they appear like mature lymphocytes and not leukemic blasts (7–9). However, immature-looking hematogones can also be present in occasional cases. Therefore, morphologic verification of an immature cell phenotype is always necessary, particularly in pediatric patients or those after chemotherapy or bone marrow transplantation.

There is a tendency for commercial laboratories to offer a blast count based on the percentages of immature cell markers without the knowledge of clinical diagnosis and morphologic correlation. The general practice is to multiply the percentage of the gated population by the percentage of immature cell markers in this particular population. For instance, if the myeloid gate accounts for 50% of the total events registered in the flow cytometric analysis and 30% of the gated population expresses CD34, the blast count is then reported as 15%. This result can be very misleading, because the immature markers, as mentioned above, may be expressed by different kinds of cells and each dot (or event) in the dot plot does not represent an intact cell. These dots may include cell debris, noncell particles, platelets, and other contaminants.

Selective Loss of One or More Cell Lineage Antigens

Selective loss of one or more cell lineage antigens on a group of lymphoid cells is also an indication of malignancy (6,10). This criterion is particularly useful for diagnosis of T-cell lymphomas because there are no clonal markers for T cells analogous to light-chain restriction for B cells. When three pan-T-cell surface markers (CD3, CD5, and CD7) are included in a study panel, the early-appearing marker (CD7) is more frequently demonstrated in the

tumor derived from an early T-cell developmental stage, whereas the late-appearing T-cell markers (CD3 and CD5) may be decreased. In contrast, the late-appearing marker (CD3) is more frequently seen in peripheral T-cell lymphomas, and the early-appearing marker (CD7) may be absent. The gradual decrease in CD7+ cells in contrast to the persistence of CD3+ cells in cases of mycosis fungoides is one of the most dramatic examples. However, one must distinguish cytoplasmic from surface CD3, because the former is a marker that appears in immature T cells.

Another indicator for the existence of a monoclonal T-cell population is the predominance of a T-cell subset, mostly CD4 and occasionally CD8. However, the minor component (CD4 or CD8) usually is not entirely absent. In addition, in viral infections, especially human immunodeficiency virus infection, CD8 will be markedly increased and CD4 will drop to a very low percentage. In Hodgkin lymphoma, in contrast, flow cytometric analysis of a lymph node may show predominantly CD4+ cells. Therefore, the selective loss of a T-cell subset alone is not diagnostic for non-Hodgkin lymphoma unless proven morphologically.

Analysis of T-Cell Receptor-V β (TCR-V β) Repertoire

Recent availability of a panel of TCR-V β antibodies that covers more than 65% of all V β domain or family provides the opportunity for identification of a monoclonal T-cell population by FC (11,12). A commercial 8-tube kit is available for this purpose. Each tube contains 3 TCR-V β antibodies. An aberrant T-cell population is first selected by T-cell marker gating (using CD3, CD4, CD5, CD7, and CD8 antibodies). This population is then submitted for TCR-V β repertoire analysis. If a single TCR-V β is expressed by more than 40% of cells in the total T-cell population, it indicates the presence of a monoclonal T-cell population (Fig. 6.2). On the other hand, if more than 70% of cells fail to react with any of TCR-V β antibodies, a possible monoclonal T-cell population beyond the area of coverage by the monoclonal antibody set should be suspected. The flow cytometric technique is comparable to the polymerase chain reaction in analyzing the V β repertoire, but it is more convenient to perform and provides quantitative results for therapeutic monitoring (11).

SELECTION OF MONOCLONAL ANTIBODY PANELS

A lymphoma or leukemia is diagnosed not by a single specific marker but by a panel of monoclonal antibodies. Therefore, the selection of a suitable monoclonal antibody panel is one of the most important steps for an accurate diagnosis of hematologic neoplasms. There have been many review articles summarizing the characteristic panels for each hematologic neoplasm (13–16). However, the state of the art is to balance between the inclusion of enough monoclonal antibodies to cover the differential diagnoses and the exclusion of excessive monoclonal antibodies to

maintain cost-effectiveness. There are many monoclonal antibodies available. They can be used to detect surface, cytoplasmic, and nuclear antigens. According to their function, these antigens can be further divided into six categories (Table 6.2).

Lineage-Associated Antigens

This category of antigens is most frequently used in a clinical setting for identifying the lineage of the tumor cells and further narrowing down the differential diagnosis. This category is further divided into B-cell, T-cell, NK-cell, and myelomonocytic cell antigens. The list of B-cell-associated antigens is rapidly expanding. The most common ones include CD10, CD19, CD20, CD22, CD23, CD24, CD79, CD138, and PCA-1. The T-cell-associated antigens include CD1, CD2, CD3, CD4, CD5, CD7, and CD8. The NK-cell-associated antigens consist of only three antigens—CD16, CD56, and CD57—although other antigens, such as CD11c, are frequently expressed by NK cells. The myelomonocyte-associated antigens include CD11b, CD11c, CD13, CD14, CD15, CD33, CD64, CD68, CD117, and myeloperoxidase. In addition, there are antibodies for identification of erythroid cells, megakaryocytes, and follicular dendritic cells.

Immature Cell Antigens

This category includes CD10, CD34, CD117, and TdT. The presence of these markers in a large number of cells usually indicates a hematologic neoplasm, except for the regenerating lymphocytes and/or myelomonocytic cells, hematogones, and dysplastic myeloid cells, which may express one or more immature cell markers.

Activation Antigens

This category is composed of CD25, CD26, CD30, CD38, CD54, CD71, and HLA-DR. These activation antigens may serve as growth factor receptors, may be involved in cell-to-cell interaction, and bind to a microenvironment (17). The activation antigens are usually present on actively proliferating tumor cells, thus conferring a poor prognosis. Some activation antigens may be associated with a particular tumor, such as CD25 in hairy cell leukemia and CD30 in anaplastic large-cell lymphoma.

Histocompatibility Antigens

Histocompatibility antigens are important in directing cell-to-cell interaction. For instance, the CD4 cells react with cells carrying HLA-II antigen, whereas the CD8 cells react with those bearing HLA-I antigen. The HLA-II antigen includes HLA-DP, HLA-DR, and HLA-DQ. Among them, HLA-DR is the most frequently used antigen for immunophenotyping. HLA-DR is an early-appearing B-cell antigen, but its presence persists into the mature stage. Therefore, if HLA-DR is demonstrated alone without other cell lineage-associated antigens, it is consistent with a stem cell leukemia or lymphoma. HLA-DR is also present on activated T cells, myeloblasts, and monoblasts, but not on promyelocytes. Its absence on promyelocytes helps to identify acute promyelocytic leukemia.

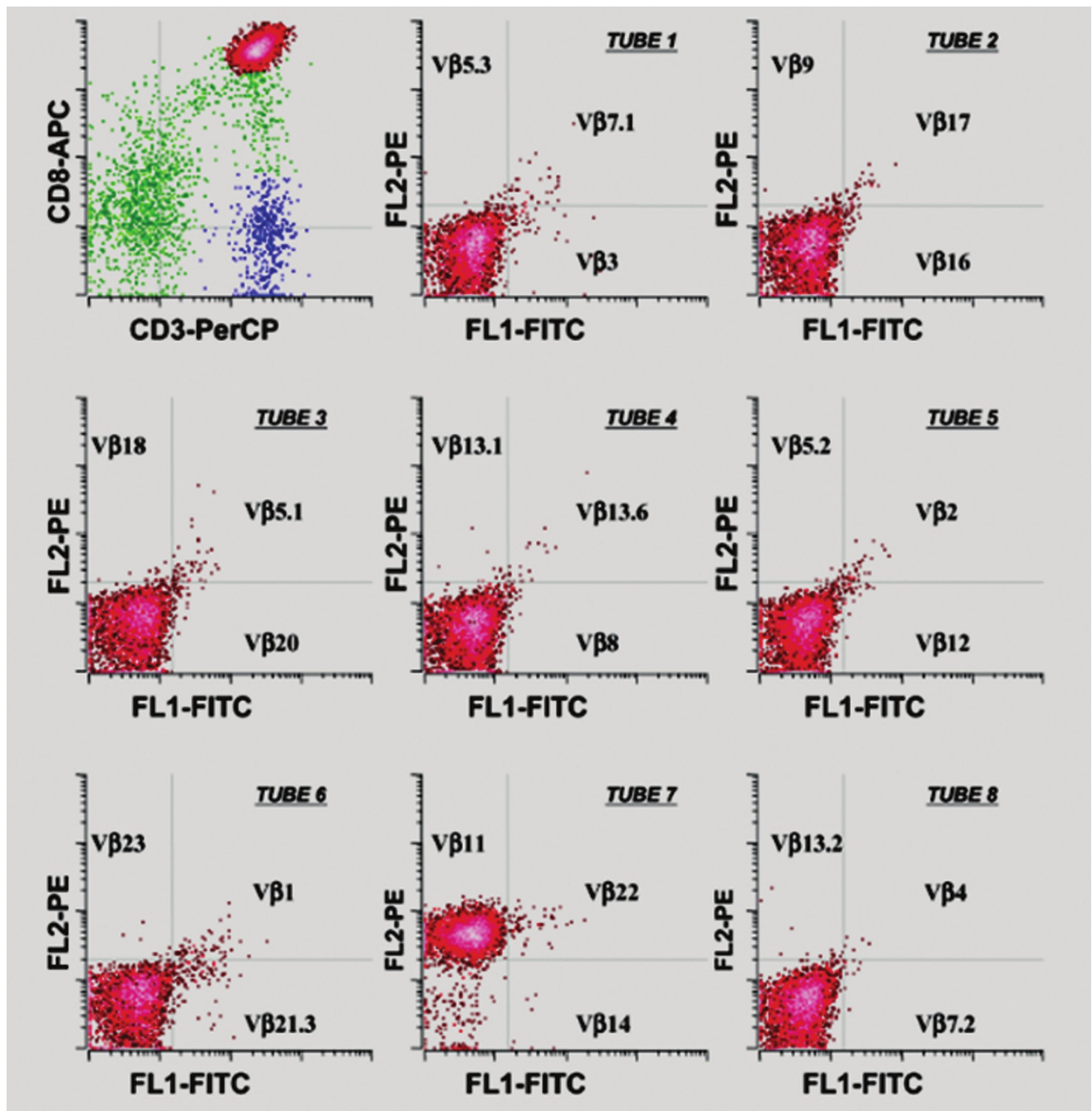


FIGURE 6.2 TCR-Vb analysis using four-color FC. The first histogram shows the gating of the aberrant T-cell population (red) with CD8 and CD3. Each of the remaining eight histograms shows the reactions of three TCR-Vb antibodies. In this setting, a positive reaction is demonstrated with the TCR-Vb11 antibody only. (From Ying L, et al. Flow-cytometric assessment of T-cell clonality in clinical specimens. *Lab Medicine* 2007;38:477–482, with permission.)

Adhesion Molecules

Many adhesion molecules have been discovered in recent years, so that the inclusion of all antigens in this category is impossible. Adhesion molecules on lymphocytes play an important role in interactions with vascular endothelium and the extracellular matrix, thus controlling lymphocyte homing and migration (18–20). In large-cell lymphomas, the expression of the lymphocyte homing receptor, CD44, is frequently demonstrated in neoplasms of stages III and

IV, but infrequently among cases of stages I and II (18). It appears that CD44 expression may influence lymphoma dissemination. Small lymphocytic lymphoma and chronic lymphocytic leukemia share the same immunophenotype except for the adhesion molecules CD11a/CD18, which are present in the former but absent in the latter (17). The presence or absence of adhesion molecules may explain why cells of small lymphocytic lymphoma stay in tissue, whereas those of chronic lymphocytic leukemia spread to

TABLE 6.2

Categories of Surface and Nuclear Antigens

Lineage-associated antigens

B cell: CD10, CD19, CD20, CD22, CD23, CD24, CD38, CD79, CD138, BCL-2, BCL-6, cyclin D1, Pax5, PCA-1, immunoglobulins (IgA, IgG, IgM, IgD, k, l)

T cell: CD1, CD2, CD3, CD4, CD5, CD7, CD8, CD43, CD45RA, CD45RO, bF1, TCRd1

NK cell: CD16, CD56, CD57, TIA-1, Gr-B

Myelocyte/monocyte: CD11b, CD11c, CD13, CD14, CD15, CD33, CD64, CD68, CD117, CD163, myeloperoxidase

Erythroid cell: glycophorin A, hemoglobin A, CD71

Megakaryocyte: CD41, CD42, CD61, factor VIII

Follicular dendritic cell: CD21, CD23, CD35

Immature cell antigens: CD10, CD34, CD117, TdT

Activation antigens: CD25, CD26, CD30, CD38, CD54, CD71, HLA-DR

Histocompatibility antigens: HLA-I, HLA-II (HLA-DP, HLA-DR, HLA-DQ)

Adhesion molecules: CD11a/CD18 (LFA-1), CD44, CD56 (NCAM), CD54 (ICAM-1), CD102 (ICAM-2), CD106 (VCAM-1), CD31 (PECAM-1)

Proliferation-associated antigens: Ki-67, PCNA

TdT, terminal deoxynucleotidyl transferase; Gr-B, granzyme-B; TIA-1, T-cell intracellular antigen; LFA, lymphocyte function–associated antigen; NCAM, neural cell adhesion molecule; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule; PECAM, platelet-endothelial-cell adhesion molecule.

the bloodstream. In addition, there are neural cell adhesion molecules, intercellular adhesion molecules (types 1 and 2), vascular-cell adhesion molecule type 1, and platelet-endothelial-cell adhesion molecule type 1.

Proliferation-Associated Antigens

The commonly known proliferation-associated antigens include Ki-67 and PCNA. Ki-67 is a nuclear antigen associated with proliferation and is expressed in all phases of the cell cycle except for the G_0 phase (21). PCNA, in contrast, is present in low concentrations in G_1 - and G_2 -M phases, but in high concentration in S phase (22). Because these antigens are expressed only on the nuclei of proliferative cells, calculation of the percentage of Ki-67– or PCNA-positive cells may give a clue to the aggressiveness of the tumor. Detection of a high percentage of Ki-67–positive cells in a low-grade lymphoma is suggestive of transformation to a high-grade lymphoma.

The approach of monoclonal antibody selection differs in different laboratories under various situations (23). There are essentially three ways to select the panels.

Standard Panel

This approach was most common in the early era of FC when the number of monoclonal antibodies was limited. The standard panel usually included representative antibodies from different cell lineage, including B cell, T cell, monocyte, and HLA-DR. B-cell antibodies include CD19, CD20, and Igs. T-cell antibodies consist of three pan-T-cell antibodies, CD3, CD5, and CD7. The monocyte marker, CD14, is frequently combined with the panleukocyte

antibody, CD45, for a gate check. However, because lymphoma and leukemia are further subclassified, as illustrated by the revised European–American Classification of Lymphoid Neoplasms and the World Health Organization classification, immunophenotyping has become increasingly complicated. A standard panel of 16 or 19 monoclonal antibodies can no longer meet the demand of the modern trend. Further expansion of the standard panel is not only unpopular under the current climate of cost containment, but also unfeasible because it needs a large sample.

A current trend of creating a computer database to facilitate interpretation of immunophenotyping for hematopoietic neoplasms by FC also falls into this category (24–27). On the basis of the computer score, a list of differential diagnoses is generated, and a correct diagnosis usually falls into one of the top four or five choices. This approach requires the use of a large panel of antibodies, yet it only narrows the range of differential diagnosis.

Two-Tiered Approach

This approach includes the use of a simple screening panel to obtain some preliminary information and, on the basis of this information, determines a specific panel (28). This approach is particularly suitable for acute leukemia. For instance, a few immature markers (e.g., TdT, CD10, CD34, and CD117) are analyzed; if one or more of them are positive, cell lineage markers are added for subclassification. Positive reactions to TdT and CD10 will direct the use of lymphoid markers for the diagnosis of acute lymphoblastic leukemia. In contrast, positive CD117 and CD34 will point to the direction of acute myeloid leukemia. Currently, a few

TABLE 6.3

Cell Specificity and Clinical Application of Common Monoclonal Antibodies

Cluster designation	Monoclonal antibodies	Cell specificity	Clinical application
CD 1a	Leu6, OKT6, T6	Thymocyte, Langerhans cells	T-ALL, T-lymphoma, histiocytosis
CD2	Leu5, OKT11, T11	E-rosette receptor	T-ALL, T-lymphoma
CD3	Leu4, OKT3, T3	T-cell receptor complex	T-ALL, T-lymphoma
CD4	Leu3, OKT4, T4	Helper/inducer T cell	Identification of T subset
CD5	Leu1, OKT1, T1	T cell, B cell subset	T-ALL, T/B lymphoma, CLL
CD7	Leu9, OKT16, 3A1	T cell receptor for IgM-Fc	T-ALL, T-lymphoma
CD8	OKT8, T8	Cytotoxic/suppressor T cell	Identification of T subset
CD10	CALLA, OKBcALLa, J5	Immature B cell and T cell	ALL, B-lymphoma
CD11b	Leu15, OKM1, Mo1	Monocyte, granulocyte, NK cell, T-suppressor cell	AML
CD11c	LeuM5, a S-HCL3,	Monocyte, B cell from HCL	AML, HCL
CD13	LeuM7, OKM13, My7	Monocyte, granulocyte	AML
CD14	LeuM3, OKM14, MY4, Mo2	Monocyte, granulocyte	AML
CD15	LeuM1, My1	Monocyte, granulocyte, Reed–Sternberg cell	Hodgkin lymphoma
CD16	Leu11	NK cell, granulocyte, macrophage	NK-cell disorder
CD19	Leu12, OKpanB, B4	B cell	B-ALL, B-lymphoma, CLL
CD20	Leu16, B1	B cell	B-ALL, B-lymphoma, CLL
CD21	CR2, OKB7, B2	Follicular dendritic cell, B cell, C3d	B-lymphoma
CD22	Leu14, OKB22, B3, a S-HCL1	B cell	B-lymphoma, HCL
CD23	B6, Leu20	B cell	B-lymphoma, CLL
CD25	IL-2, OKT26a, Tac	IL-2 receptor on T cell (Tac antigen)	HCL, adult T-cell leukemia
CD30	Ki-1, BerH2	Reed–Sternberg cell, activated T or B cell	Hodgkin lymphoma, anaplastic large-cell lymphoma
CD33	LeuM9, My9	Monocyte, granulocyte	AML
CD34	HPCA-1, My10	Hematopoietic progenitor cell	Acute leukemia
CD38	Leu17, OKT10, T10	Plasma cell, activated T or B cell	Myeloma
CD41	J15	Platelet GPIIb/IIIa	Megakaryoblastic leukemia
CD42a,b	HPL14, AN51, 10P42	Platelet GPIX and GPIb	Megakaryoblastic leukemia
CD43	MT-1, Leu22, L60	T cell, B cell subset	T- or B-cell lymphomas
CD45	HLE-a, LCA	All leukocytes	Lymphomas, leukemias
CD45RA	MT-2	T cell, B cell subset	Follicular lymphoma
CD45RO	UCHL1	T cell, B cell, monocyte, granulocyte	T-lymphoma
CD56	Leu19, NKH-1	NK cell	NK cell disorder
CD57	Leu7, HNK-1	NK cell, T cell subset	NK cell disorder
CD61	10P61, VI-PL2	Platelet GPIIIa	Megakaryoblastic leukemia
CD64	FcγP1, gp75	Monocyte	Monocytic disorder
CD68	KP1	Monocyte, histiocyte	Monocytic/histiocytic tumors

(continued)

TABLE 6.3 (continued)

Cell Specificity and Clinical Application of Common Monoclonal Antibodies

CD74	LN2	B cell, monocyte	B-lymphoma
CDw75	LN1	B cell, T cell subset	B-lymphoma
CD79a	HM47, HM56, JAB117	B cell	B-lymphoma
CD79b	SN8, B29/123, CH3-1	B cell	B-lymphoma
CD103	HML-1, B-ly7	B cell	HCL
CD117	C-kit, stem cell factor receptor	Hematopoietic stem cell	AML
CD123	IL-3 receptor	Mast cell/basophil, DC2 cells B cell subset	Mast cell disease, hairy cell leukemia
CD138	B-B4, 1D4, F59-2E9, M115	B cell, plasma cell	B-lymphoma. Myeloma
CD235a	Glycophorin A	Erythroid series	Erythroleukemia
	FMC-7	B cell	PLL, HCL, B-lymphoma
	HLA-DR	B cell, activated T cell, myeloblast, monoblast	B cell neoplasms
	PCA-1	Plasma cell, monocyte, granulocyte	Myeloma
	TCR-1, bF-1, WT31	T cell	T-lymphoma/leukemia
	TCR-d1, TCS1, antid	T cell	T-lymphoma/leukemia

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; HCL, hairy cell leukemia; IL-2, interleukin 2; NK, natural killer; PLL, prolymphocytic leukemia; Tr, transferrin.

core panels are suggested for the differential diagnosis of small B-cell lymphomas. Examples are panels consisting of CD20, CD10, CD23, and k and l light chains (29) or CD5, CD10, and CD23 (30) or double staining for CD23 and FMC-7 (31). In this approach, additional antibodies can be added later for a final diagnosis. Although this approach is economical in the optimal use of monoclonal antibodies, it is time consuming. Unless the screening and corroborative tests are done on the same day, this approach is generally not acceptable clinically.

Targeted Approach

This is the most efficient way to select a panel of monoclonal antibodies (28). It requires a morphologist to review the blood smear, bone marrow aspirate, or frozen section to make a preliminary diagnosis, and a monoclonal antibody panel will then be set up accordingly. If no specimen is available for examination, a preliminary clinical diagnosis should be obtained to determine the panel. With this approach, usually only six to eight monoclonal antibodies are needed. The remaining part of the specimen should be

TABLE 6.4

Minimal Monoclonal Antibody Panels for Diagnosis of Hematologic Neoplasms

Panel	Antibodies
B-cell lymphoma/chronic lymphocytic leukemia	k/l, CD5/CD19, CD23, CD10, FMC-7
T-cell lymphoma/leukemia	CD3, CD4, CD5, CD7, CD8, CD25, CD30
Hairy cell leukemia	k/l, CD11c/CD22, CD25, CD103, FMC-7
NK lymphoma/leukemia	CD2, CD3, CD4, CD8, CD16, CD56, CD57
ALL	TdT, CD7, CD10, CD19, CD34, C _m , k/l
AML	MPO, CD7, CD13, CD14, CD33, CD34, CD64, CD117, HLA-DR
AML-M6 (erythroleukemia)	AML panel plus glycophorin A
AML-M7 (megakaryoblastic leukemia)	AML panel plus CD41, CD42b, CD61
Myeloma/macroglobulinemia	Surface and cytoplasmic k/l, CD5, CD19, CD38, CD56, CD138

MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase; NK, natural killer; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia.

saved in the refrigerator for additional monoclonal antibody testing, in the event that the preliminary diagnosis is incorrect or further subclassification is desired. However, Nguyen et al. (32) found that morphologic misinterpretation often occurred, leading to incorrect panel selection. These authors suggested setting up two large standard panels, designated blood/bone marrow/spleen panel and tissue/fluid panel, respectively.

Since the early 1980s, thousands of monoclonal antibodies specific for leukocyte-differentiation antigens have been developed. These antibodies are categorized into different functional groups, and those that are reacting to the same epitope are assigned the same cluster designation or differentiation (CD). At the 8th International Workshop on Leukocyte Differentiation Antigens held in Adelaide, Australia, in December 2004, the last CD was CD339 (33). These antibodies have been used mainly on fresh and appropriately frozen cells. However, there has been an increasing number of newly developed antibodies that are reactive with antigens in fixed paraffin-embedded sections (34,35) (see Table 3.2). These antibodies are most helpful in morphologic correlation with immunophenotypes and in retrospective studies. The monoclonal antibodies that are commonly used for immunophenotyping of lymphomas and leukemias are summarized in Table 6.3. The minimal monoclonal antibody panels used in different types of lymphoma and leukemia are listed in Table 6.4.

REFERENCES

1. Rothe G, Schmitz G, Adort D, et al. Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies. *Leukemia*. 1996;10:877–895.
2. Taylor CR. Result of multiparameter studies of B-cell lymphomas. *Am J Clin Pathol*. 1979;72(suppl):670–686.
3. Samoszuk MK, Krailo M, Yan QH, et al. Limitations of numerical ratios for defining monoclonality of immunoglobulin light chains in B-cell lymphomas. *Diagn Immunol*. 1985;3:133–138.
4. Sun T, Susin M, Desner M, et al. The clonal origin of two cell populations in Richter's syndrome. *Hum Pathol*. 1990;21:722–728.
5. Sun T, Susin M. A practical approach to immunophenotyping of lymphomas: comparison of immunohistologic and immunocytologic techniques. *Ann Clin Lab Sci*. 1987;17:14–16.
6. Picker LJ, Weiss LM, Medeiros LJ, et al. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. *Am J Pathol*. 1987;128:181–201.
7. Longacre TA, Foucar K, Crago S, et al. Hematogones: a multiparameter analysis of bone marrow precursor cells. *Blood*. 1989;73:543–552.
8. Davis RD, Longacre TA, Cornbleet J. Hematogones in the bone marrow of adults: immunophenotypic features, clinical settings, and differential diagnosis. *Am J Clin Pathol*. 1994;102:202–211.
9. Leitenberg D, Rapeport JM, Smith BR. B-cell precursor bone marrow reconstitution after bone marrow transplantation. *Am J Clin Pathol*. 1994;102:231–236.
10. Sun T, Ngu M, Henshall J, et al. Marker discrepancy as a diagnostic criterion for lymphoid neoplasms. *Diagn Clin Immunol*. 1988;5:393–399.
11. Langerak AW, van den Beemd R, Wolvers-Tettero ILM, et al. Molecular and flow cytometric analysis of the Vb repertoire for clonality assessment in mature TCRab T-cell proliferations. *Blood*. 2001;98:165–173.
12. van den Beemd R, Boor PPC, van Lochem EG, et al. Flow cytometric analysis of the Vb repertoire in healthy controls. *Cytometry*. 2000;40:336–345.
13. Thakhi A, Etinger M, Myles J, et al. Flow cytometric immunophenotyping of non-Hodgkin's lymphomas and related disorders. *Cytometry*. 1996;25:113–124.
14. Jennings CD, Foon KA. Recent advances in flow cytometry, application to the diagnosis of hematologic malignancy. *Blood*. 1997;90:2863–2892.
15. Ward MS. The use of flow cytometry in the diagnosis and monitoring of malignant hematological disorders. *Pathology*. 1999;31:382–392.
16. Knowles DM. Immunophenotypic markers useful in the diagnosis and classification of hematopoietic neoplasms. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:93–226.
17. Freeman AS, Nadler LM. Immunologic markers in non-Hodgkin's lymphoma. *Hematol Oncol Clin North Am*. 1991;5:871–889.
18. Horst E, Meijer CJML, Radaszkiewicz T, et al. Adhesion molecules in the prognosis of diffuse large-cell lymphoma. Expression of a lymphocyte homing receptor (CD44), LFA-1 (CD11a/18), and ICAM-1 (CD54). *Leukemia*. 1990;4:595–599.
19. Pals ST, Horst E, Ossekoppels GJ, et al. Expression of lymphocyte homing receptor as a mechanism of dissemination in non-Hodgkin's lymphoma. *Blood*. 1989;73:885–888.
20. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood*. 1994;84:2068–2101.
21. Gendes J, Lemke H, Baisch H, et al. Cell cycle analyses of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol*. 1984;133:1710–1715.
22. Garcia RL, Coltrera MD, Gown AM. Analysis of proliferative grade using anti-PCNA/cyclin monoclonal antibodies in fixed embedded tissues. *Am J Pathol*. 1989;134:733–739.
23. Stewart CC, Behm FG, Carey JL, et al. U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: selection of antibody combinations. *Cytometry*. 1997;30:231–235.
24. Verwer B, Terstappen L. Automatic lineage assignment of acute leukemia by flow cytometry. *Cytometry*. 1993;14:862–875.
25. Diamond LW, Ngyen DT, Andreeff M, et al. A knowledge based system for the interpretation of flow cytometry data in leukemias and lymphomas. *Cytometry*. 1994;17:266–271.
26. Thews O, Thews A, Huber C, et al. Computer assisted interpretation of flow cytometry data in hematology. *Cytometry*. 1996;23:140–149.
27. Nguyen AN, Milam JD, Johnson KA, et al. A relational database for diagnosis of hematopoietic neoplasms using immunophenotyping by flow cytometry. *Am J Clin Pathol*. 2000;1113:95–106.
28. Wood BL, Arroz M, Barnett D, et al. 2006 Bethesda International Consensus Recommendations on the Immunophenotypic Analysis of Hematolymphoid Neoplasia by Flow Cytometry: optimal reagents and reporting for the flow cytometric diagnosis of hematopoietic neoplasia. *Cytometry Part B*. 2007;728:S14–S22.
29. Kurtin PJ, Hobday KS, Ziesmer S, et al. Demonstration of distinct antigenic profiles of small B-cell lymphomas by paraffin section immunochemistry. *Am J Clin Pathol*. 2001;115:136–142.



30. Kaleem Z, White G, Wollmer RT. Critical analysis and diagnostic usefulness of limited immunophenotyping of B-cell non-Hodgkin lymphomas by flow cytometry. *Am J Clin Pathol*. 2001;115:136–142.
31. Garcia DP, Rooney MT, Ahmad E, et al. Diagnostic usefulness of CD23 and FMC-7 antigen expression patterns in B-cell lymphoma classification. *Am J Clin Pathol*. 2001;115:258–265.
32. Nguyen D, Diamond LW, Braylan RC. *Flow Cytometry in Hematopathology: A Visual Approach to Data Analysis and Interpretation*. Totowa, NJ: Humana Press; 2003.
33. Zola H, Swart B, Nicholson I, et al. CD molecules 2005: human cell differentiation molecules. *Blood*. 2005;106:3123–3126.
34. Perkins SL, Kjeldsberg CR. Immunophenotyping of lymphomas and leukemias in paraffin-embedded tissues. *Am J Clin Pathol*. 1993;99:363–373.
35. Dabbs D. *Diagnostic Immunohistochemistry. Theranostic and Genomic Applications*. 3rd ed. Philadelphia, PA: Churchill Livingstone; 2010.

CASE 1

Chronic Myelogenous Leukemia

CASE HISTORY

A 59-year-old man was admitted to the hospital because of marked leukocytosis found incidentally on routine work-related physical examination. He was otherwise asymptomatic. He denied having fevers, chills, night sweats, weight loss, and other constitutional symptoms. Physical examination on admission showed no hepatosplenomegaly and no lymphadenopathy. He is a radiation health safety officer and was estimated to have 25 rads lifetime exposure. His half sister died of leukemia at young age.

Peripheral blood examination revealed a total leukocyte count of 42,300/mL with 56% segmented neutrophils, 9% bands, 2% metamyelocytes, 5% myelocytes, 1% blasts, 19% lymphocytes, 3% monocytes, 2% eosinophils, and 3% basophils. His hematocrit was 50.6%, hemoglobin 16.4 g/dL, and platelets 463,000/mL.

A bone marrow biopsy showed 0.5% myeloblasts, 2.3% promyelocytes, 21.8% myelocytes, 8% metamyelocytes, 15% bands, 27% segmented neutrophils, 1.5% monocytes, 4.8% eosinophils, 4.8% basophils, 1.8% pronormoblasts, 1.3% basophilic normoblasts, 2.8% polychromatic normoblasts, and 8.3% orthochromatic normoblasts. The M/E ratio was 6:1. No myelodysplastic changes were demonstrated.

A core biopsy showed 90% cellularity with a widened cuff of immature myeloid cells along the bony trabeculae. The cellular component is predominantly myeloid cells. However, trilineage hematopoiesis was still present. A diagnosis of chronic myelogenous leukemia (CML) was established by flow cytometry (FC) and fluorescence in situ hybridization (FISH).

The patient was treated promptly with Gleevac. In a 3-month follow-up, the total leukocyte count returned to 4,850/mL with normal hematocrit, hemoglobin, and platelets. FISH analysis revealed no fusion signals. A bone marrow transplant is planned for the patient.

FLOW CYTOMETRY FINDINGS

Flow cytometric analysis of the bone marrow showed myeloperoxidase 78%, CD13-CD33 79%, HLA-DR 20%, CD34 13%, CD117 43%, CD14 0%, and CD7 0% (Fig. 6.1.1).

MOLECULAR GENETICS

FISH for breakpoint cluster region/Ableson 1 (BCR-ABL1) was performed, which showed fusion signals in 79% of cells.

DISCUSSION

CML was first reported in two patients in 1845, but it was not until 1960 that the association of CML with the Philadelphia chromosome (Ph) was identified (1). The karyotype of t(9;22)(q34;q11.2) was reported in 1973, and the molecular characterization of the BCR-ABL fusion gene was established in the 1980s.

CML is now recognized as a clonal myeloproliferative neoplasm that originates in a hematopoietic stem cell. Therefore, it involves not only the myeloid cells but also monocytes, erythrocytes, megakaryocytes, and lymphocytes. Its stem cell origin is evidenced by the occurrence of blasts of various cell lineages during the blast crisis and by the demonstration of BCR-ABL fusion products in different kinds of cells (2–5). This is the first hematologic neoplasm in which the association between cytogenetic aberration and leukemogenesis is established. It is one of the most common leukemias, accounting for 15% of leukemias in adults (2).

Morphology

Clinically, CML is divided into three phases: chronic, accelerated, and blast, which can be identified through morphology. In the chronic phase (CP), the peripheral blood shows leukocytosis, usually over 50,000/mL and most cases exceed 100,000/mL (6,7). The leukocytes are mainly composed of granulocytes of various stages, from myeloblasts to segmented neutrophils, but the major population is composed of myelocytes and segmented neutrophils (Fig. 6.1.2). This phenomenon is sometimes referred to as myelocyte bulge and is characteristic of CML. Peripheral basophilia, in addition to granulocytosis, is probably the most important finding for a morphologic diagnosis of CML because it helps to distinguish reactive granulocytosis. However, the absence of basophilia does not exclude CML. Eosinophilia is also a common feature in CML, but it can also be seen in allergy and many other reactive conditions, so its presence is not specific.

The blast count in the CP is <3%, and the percentage of blasts and promyelocytes combined is <10% (7). The percentage of monocytes is usually below 3%, but, due to the high leukocyte count, an absolute monocytosis may be present (5). The platelet count is usually elevated, and its morphology is often normal. In the minority of cases, giant platelets or platelets with decreased or absent granules may be present. Most patients have mild anemia, but the red cell morphology is essentially normal. Nucleated red blood cells may be detected in the peripheral blood in a small number of cases.

The bone marrow features are similar to those of the peripheral blood with a wide spectrum of myeloid cells

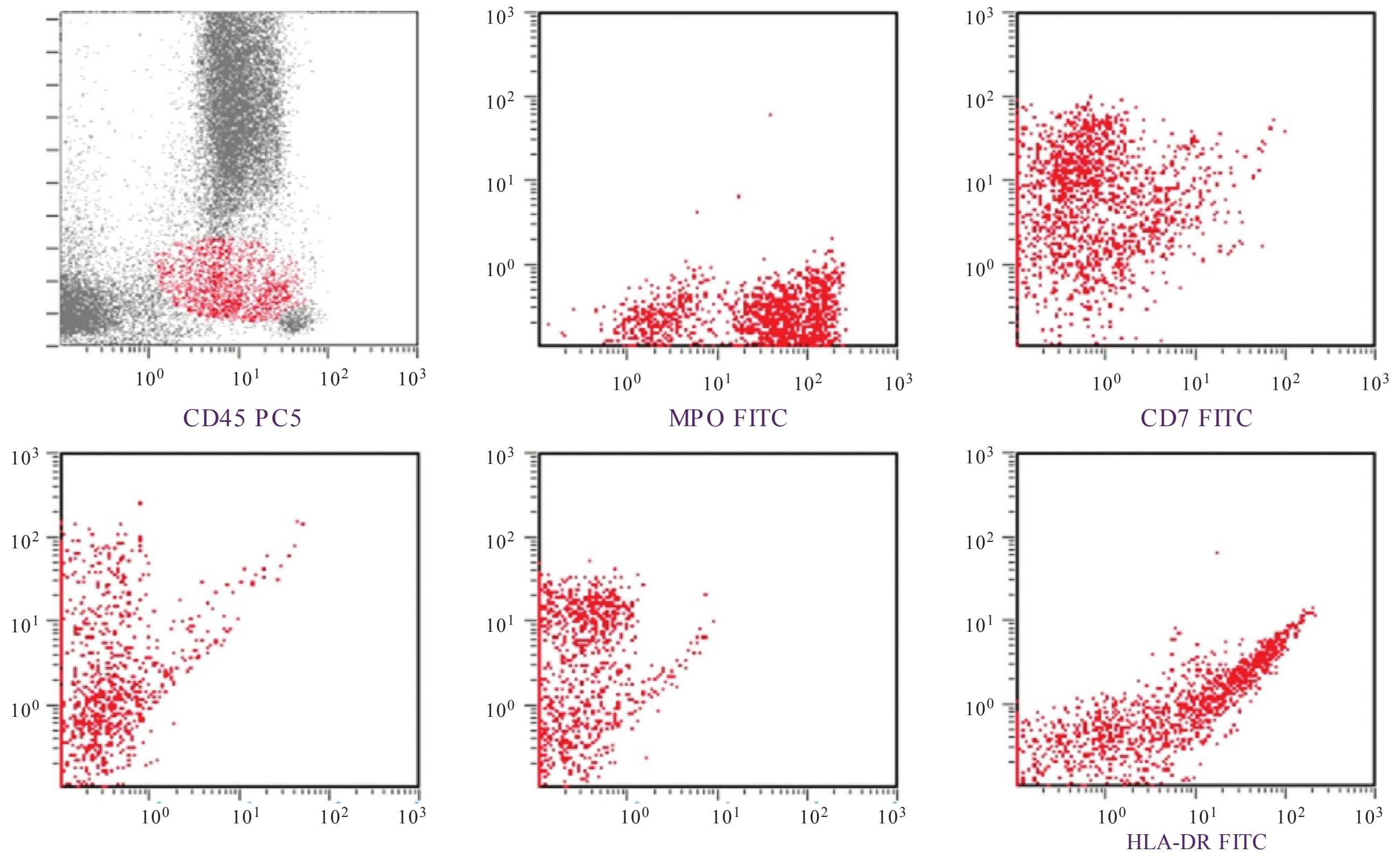


FIGURE 6.1.1 Flow cytometric analysis shows positive myeloperoxidase and CD13-CD33; partially positive CD34, CD117, and HLA-DR reactions; but negative CD7 reaction. FITC, fluorescein isothiocyanate; PE, phycoerythrin; MPO, myeloperoxidase.

(Fig. 6.1.3) (5–7). However, these cells are usually more immature than those in the blood, showing more promyelocytes and myelocytes. Blasts are usually <5% in the bone marrow. Myelodysplastic changes are not seen in the CP. Megakaryocytes are increased and are characteristically

microcytic and hypolobated (Figs. 6.1.4 and 6.1.5). In some cases, megakaryocytic hyperplasia is so prominent that some authors designated it Ph-positive essential thrombocythemia (7). The degree of myelofibrosis is usually proportional to megakaryocytosis, and reticulin fiber is increased

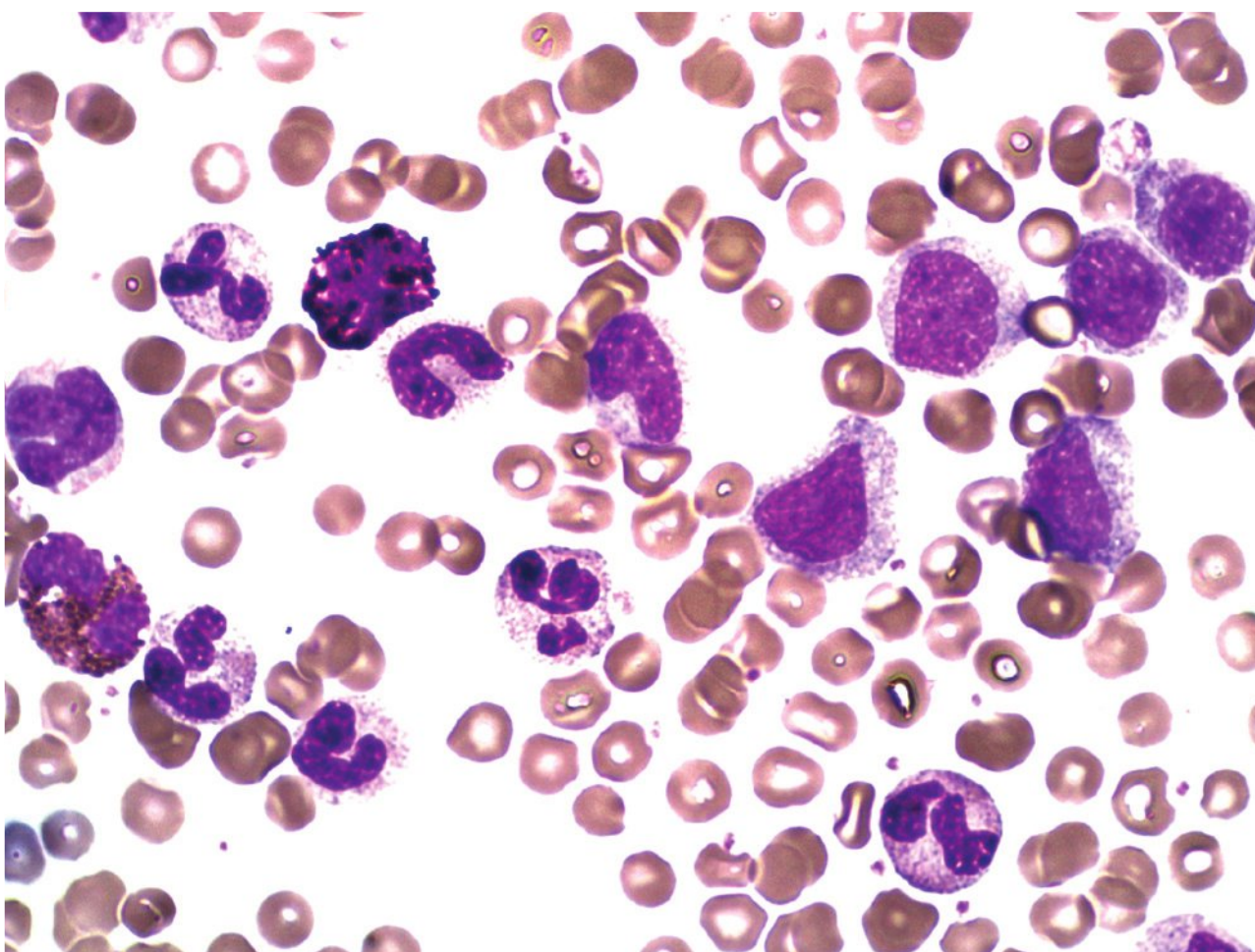


FIGURE 6.1.2 Peripheral blood smear from a patient with CP of CML shows predominantly myelocytes and segmented neutrophils. A basophil and an eosinophil are also seen. Wright-Giemsa, 100× magnification.

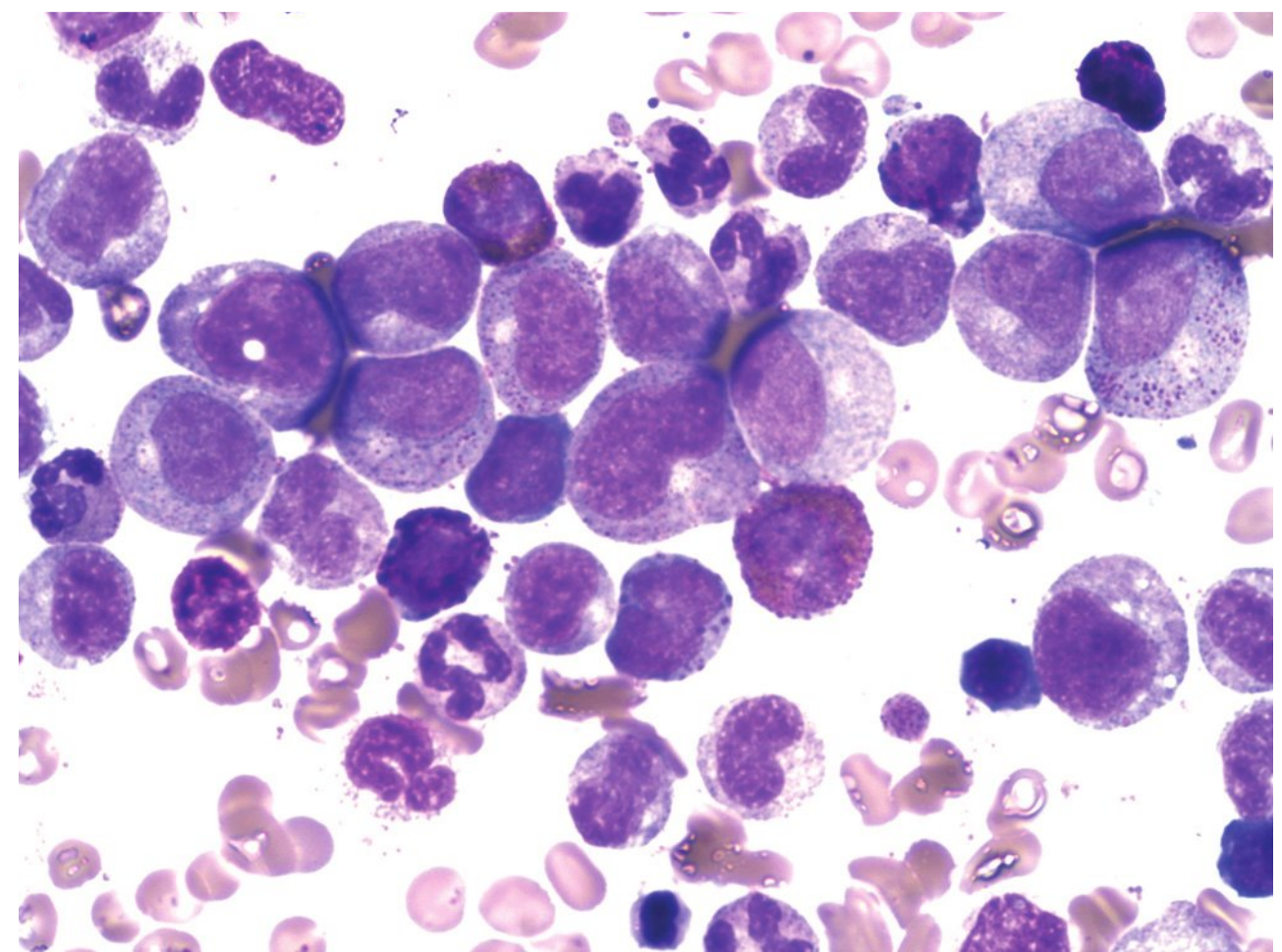


FIGURE 6.1.3 Bone marrow aspirate from a patient with CP of CML shows predominantly promyelocytes and myelocytes with a few eosinophils and basophils. Wright-Giemsa, 100× magnification.

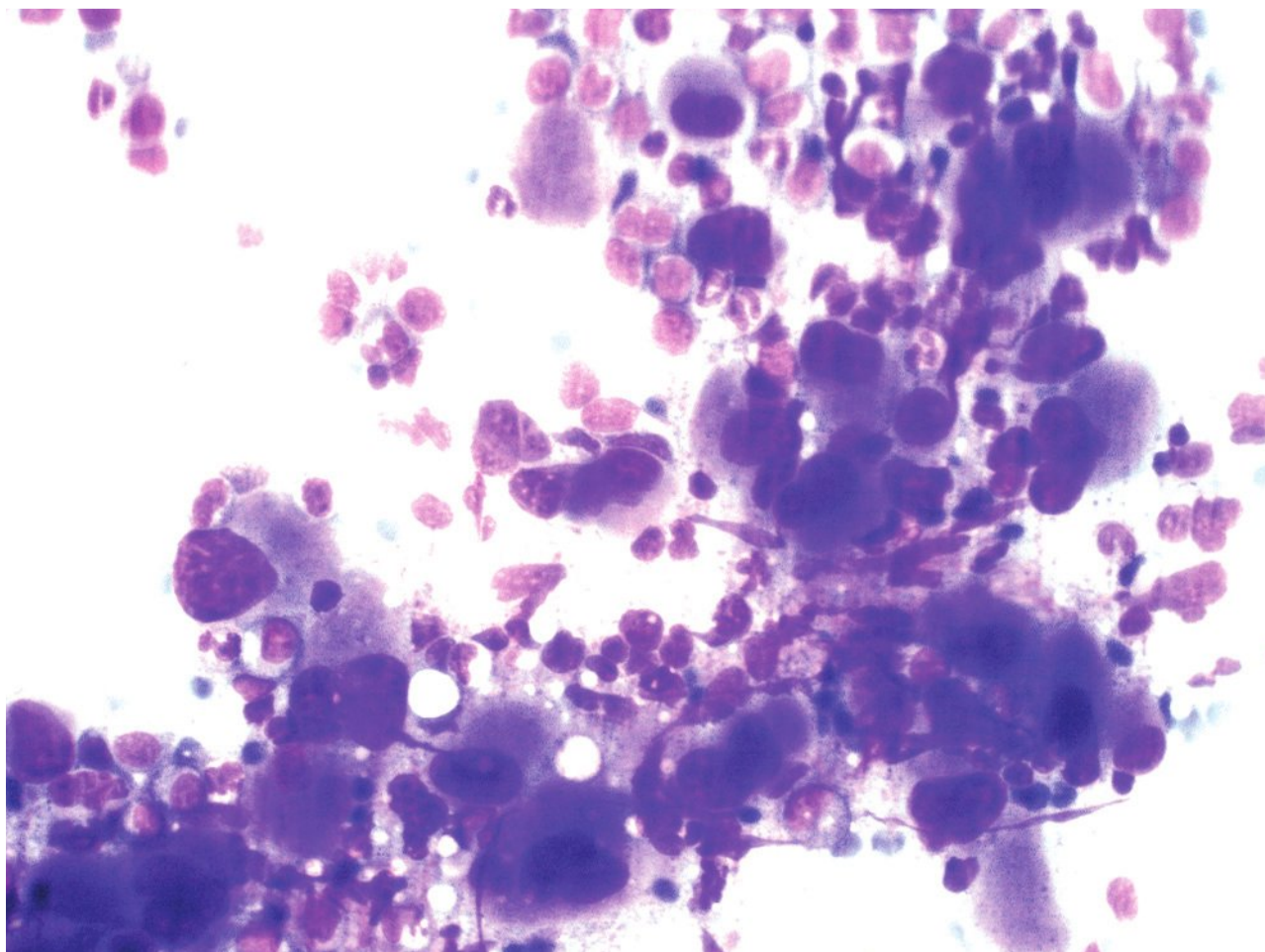


FIGURE 6.1.4 Bone marrow aspirate from a patient with CML shows a large cluster of megakaryocytes. Most megakaryocytes are mononucleated or hypolobated microcytic forms. Wright-Giemsa, 100× magnification.

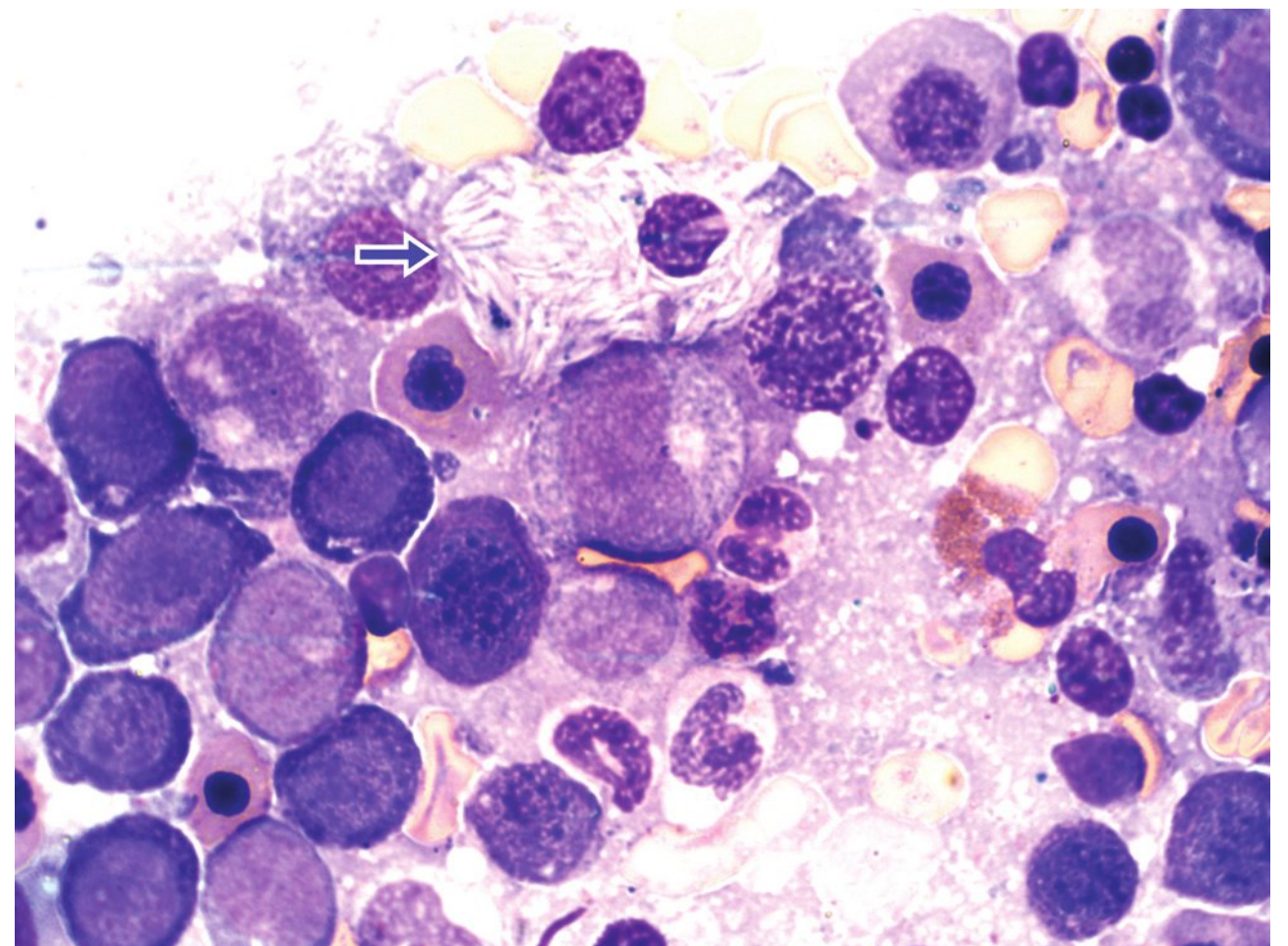


FIGURE 6.1.6 Bone marrow aspirate from a patient with CML reveals a pseudo-Gaucher cell (arrow). 100× magnification.

in up to 40% of CML patients (5). Erythrocyte precursors are generally decreased, resulting in an M/E ratio as high as 10:1. Sea-blue histiocytes and pseudo-Gaucher cells (Fig. 6.1.6) are frequently present because of an increase of cell turnover. The bone marrow biopsy shows marked hypercellularity with granulocytosis and megakaryocytosis (Fig. 6.1.7). The immature myelocytes normally reside along the bony trabeculae and form a 2- to 3-cell layer. In CML cases, the paratrabeular cuff of immature myelocytes may become widened up to a 5- to 10-cell layer (5).

The progress from CP to accelerated phase (AP) is indicated by one or more of the following criteria as defined by the 2001 World Health Organization (WHO) classification (5): (i) a blast count in the peripheral blood and/or bone marrow between 10% and 19% (Fig. 6.1.8);

(ii) peripheral blood basophils 20% or above; (iii) persistent thrombocytopenia ($<100,000/\text{mL}$) unrelated to therapy or persistent thrombocytosis ($>1,000,000/\text{mL}$) unresponsive to therapy; (iv) increasing spleen size and increasing leukocyte count unresponsive to therapy; and (v) cytogenetic evidence of clonal evolution, that is, additional chromosome abnormalities besides Ph. Marked myelodysplasia and prominent megakaryocytic proliferation with microcytic and hypolobated forms are also suggestive of AP. However, they have not yet been established as independent indicators of AP. In addition, nucleated erythrocytes are more frequently seen in the peripheral blood, and reticulin or collagen fibrosis is more prominent in the bone marrow than in CP.

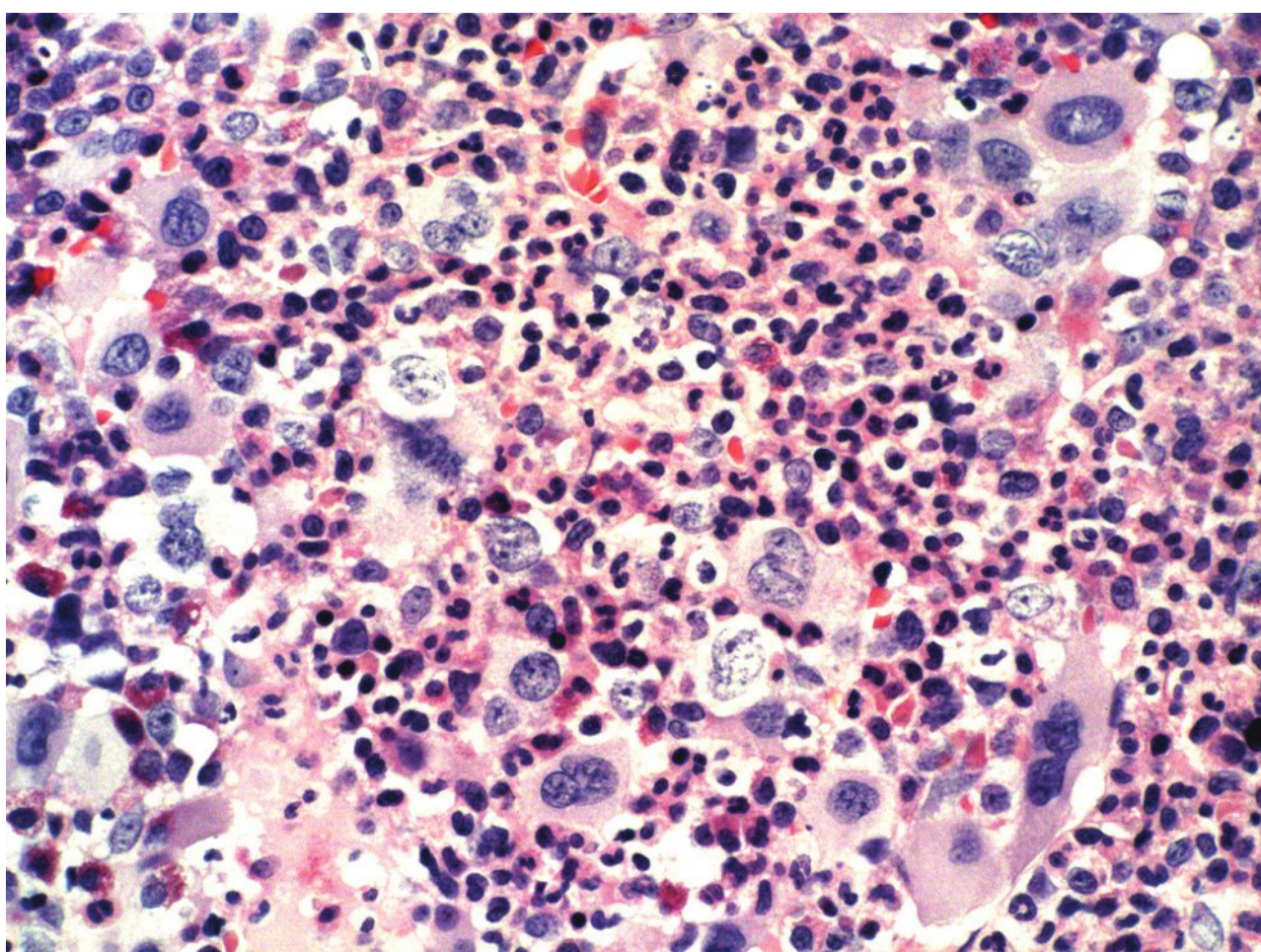


FIGURE 6.1.5 Bone marrow biopsy from a patient with CML reveals megakaryocytic proliferation. Hematoxylin and eosin, 40× magnification.

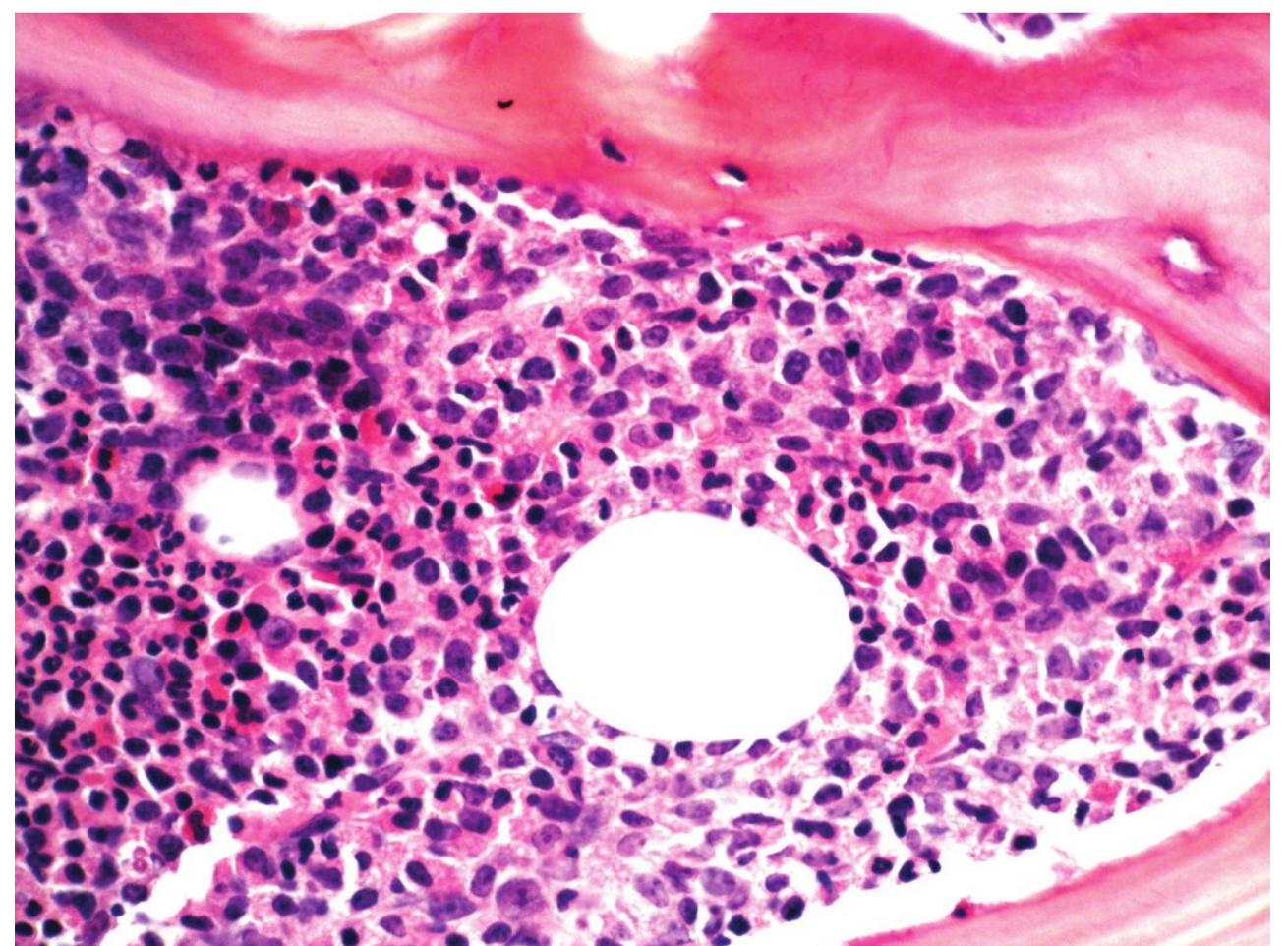


FIGURE 6.1.7 Bone marrow biopsy from a patient with CP of CML shows widening of the paratrabeular cuff of immature myeloid cells. Note that a core of mature granulocytes is separated by the immature cell layer from the bony trabecula. Hematoxylin and eosin, 40× magnification.

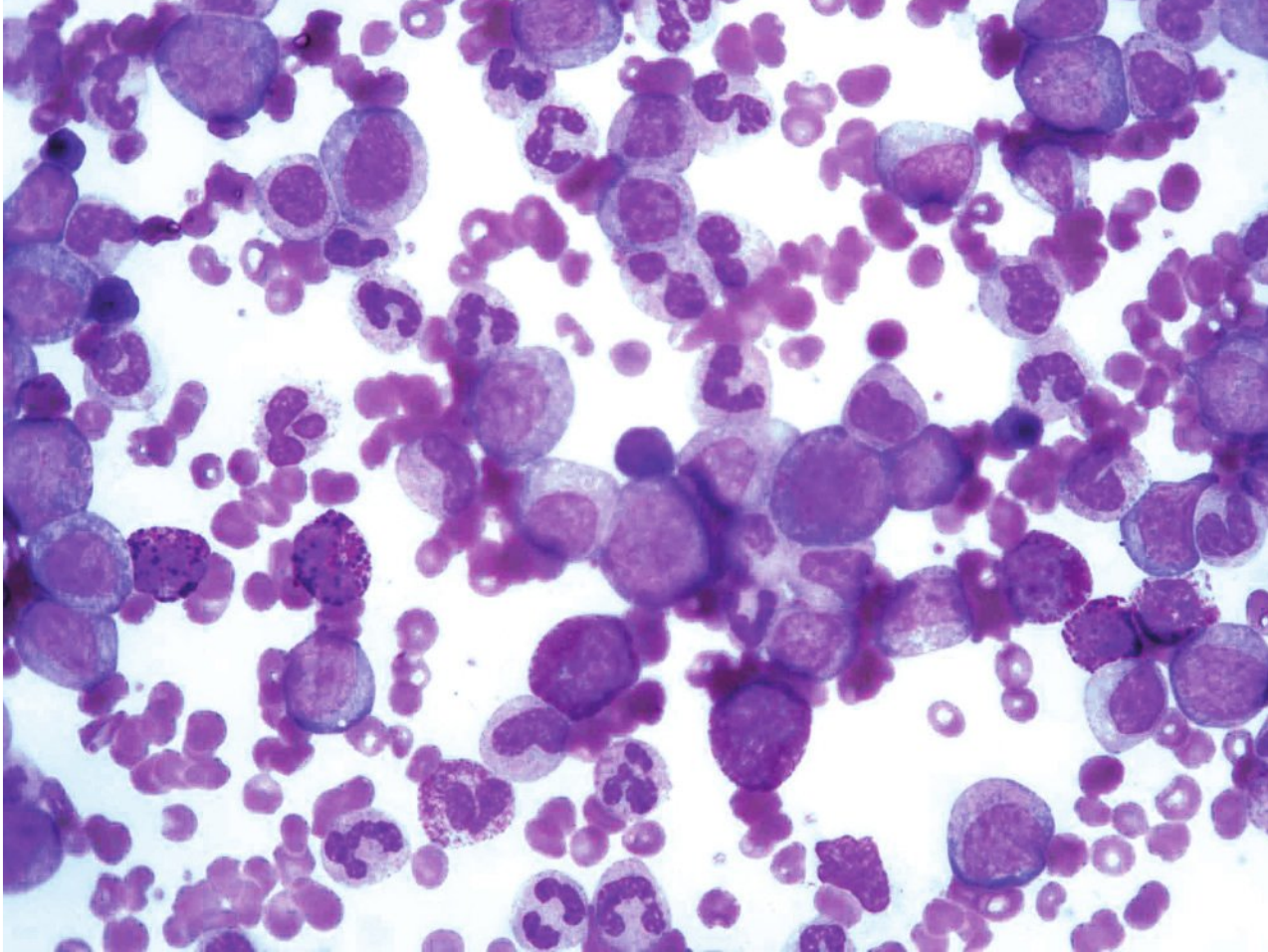


FIGURE 6.1.8 Peripheral blood from a patient with AP CML reveals an increased number of blasts with the presence of eosinophils and basophils. Wright–Giemsa, 100× magnification.

In the 2008 WHO classification, it is suggested that criteria (iii) to (v) are more likely to be associated with transition from CP to AP, while criteria (i) and (ii) more frequently indicate a transition between AP and blast phase (BP) (8). However, the European LeukemiaNet group changes the blast count in blood and bone marrow to 15% to 29% and the blast cells plus promyelocytes in blood and bone marrow to more than 30% (9). Thrombocytosis unresponsive to therapy, increasing spleen size and increasing leukocyte count unresponsive to therapy, and cytogenetic evidence of clonal evolution are not included in the diagnostic criteria by this group (9).

BP is defined as the presence of 20% or more blasts in the peripheral blood and/or bone marrow (Fig. 6.1.9) (4,5). However, BP is also indicated when large clusters of blasts are demonstrated in the bone marrow biopsy (Fig. 6.1.10) (5). Under unusual conditions, high blast count

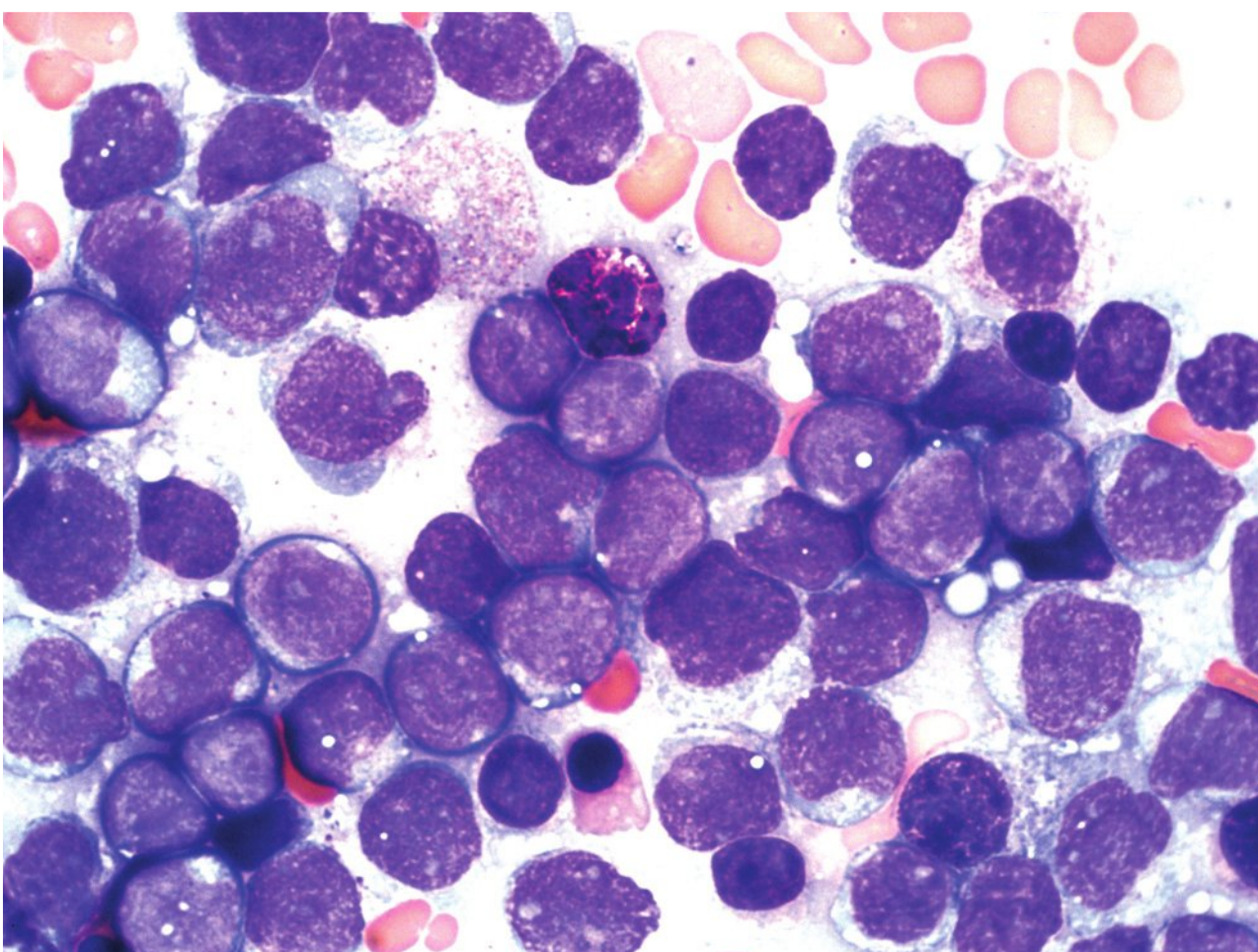


FIGURE 6.1.9 Bone marrow aspirate from a patient with BP CML shows nearly total replacement of the normal hematopoietic cells by myeloblasts. A basophil and a normoblast are also present. Wright–Giemsa, 100× magnification.

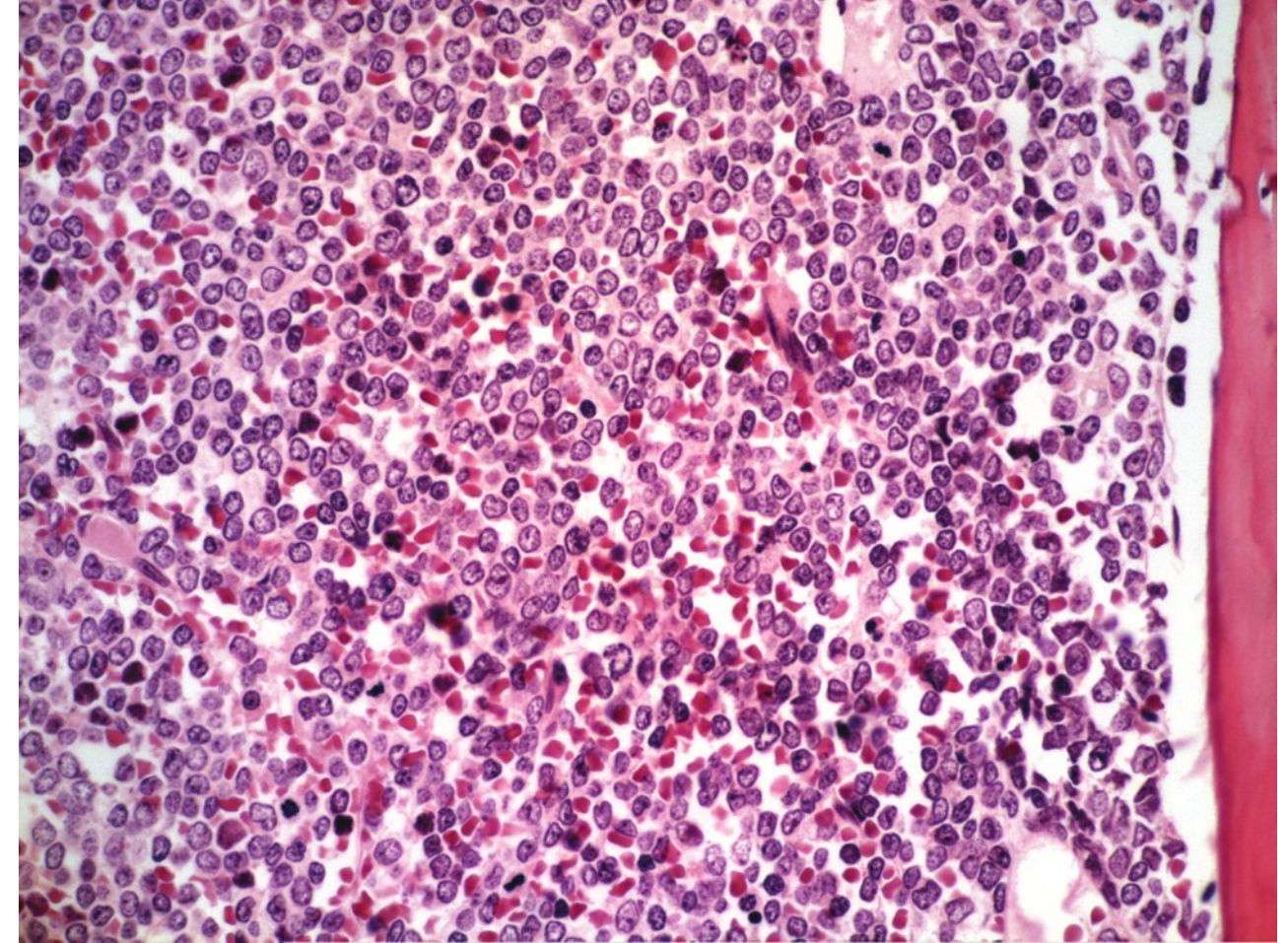


FIGURE 6.1.10 Bone marrow biopsy from a patient with BP CML shows total replacement of normal hematopoietic cells by the blasts. Hematoxylin and eosin, 40× magnification.

is not demonstrated in the peripheral blood or bone marrow, but extramedullary blast proliferation is present (10). This phenomenon was considered to be a predisposing condition of blast crisis, but it is now defined as one of the manifestations of BP (5). The 2008 WHO classification offers no modification of the 2001 WHO criteria (8), but the European LeukemiaNet group changes the cutoff of blast count in peripheral blood and bone marrow from 20% to 30% (9).

In BP, basophilia is still present, but cytopenia occurs in cell lines other than myeloid. Myelodysplastic changes become more prominent in BP than in AP. About two thirds of the cases are of myeloblastic crisis and one third lymphoblastic. However, monoblastic, megakaryoblastic, promyelocytic, erythroblastic, and multilineage blastic crises have also been reported. The identification of the blastic lineage frequently requires immunophenotyping. In a full-blown case of CML with blast crisis, it is almost impossible to distinguish it from acute myeloid leukemia morphologically, but a detailed clinical history may help. In most cases, however, CML can be distinguished from acute myeloid leukemia with multiple parameters (Table 6.1.1).

Immunophenotyping

Immunophenotyping does not play an important role in the initial diagnosis or in therapeutic monitoring. A definitive diagnosis and follow-up of the patients depend on the demonstration of t(9;22) or the BCR-ABL1 fusion product.

FC may demonstrate a myeloid population with positive CD13, CD15, CD33, and myeloperoxidase (5). Except in BP, lymphoid and monocyte antigens are generally negative. This immunophenotype is not specific because it cannot distinguish CML from leukemoid reaction. However, immunophenotype can help to distinguish various phases of CML. A flow cytometric study showed that the range of CD34-positive cells is 0% to 26% in CP, 6% to 64% in AP, and 27% to 97% in BP (11). An immunohistochemical study revealed the ranges of CD34-positive cells to be 0.1% to 1.1% in CP, 2.8% to 10.0% in AP, and 0.6% to 98% in BP (12).

TABLE 6.1.1

Comparison of Acute and Chronic Myeloid Leukemias

	Acute myeloid leukemia	Chronic myeloid leukemia
Peripheral blood	Leukemic hiatus*	Broad spectrum of granulocytes
Basophilia	Absent	Present
Platelets	Decreased	Normal or increased in early stage
Anemia	Present	Absent in early stage
Bone marrow	Blasts > 20%	Blasts < 20% except for blast crisis
Philadelphia chromosome	Absent	Present
BCR-ABL1 fusion product	Absent	Present
CD34/CD117	Markedly increased	Moderately increased

*Leukemic hiatus, presence of blasts and mature granulocytes without the presence of intermediate forms

However, due to the small sample size, the difference between the AP group and BP group was not statistically significant. CD117 is also positive in CML cases, proportional to the blast count. However, no systematic study of CD117 in CML has been reported.

Immunophenotyping is most useful in the distinction between different blasts. CD34 may identify both myeloblasts and lymphoblasts, but CD117 is positive only in myeloblasts. Lymphoblasts are positive for terminal deoxynucleotidyl transferase and CD10; the latter is mainly seen in B lymphoblasts. B and T lymphoblasts can be readily distinguished by B-cell (e.g., CD19, CD20, CD79a) and T-cell (e.g., CD3, CD7) markers. This distinction is very important because the treatment and prognosis of patients with myeloblast crisis and lymphoblast crisis are markedly different. The monoblasts are usually identified by cytochemical staining with nonspecific esterase, but they are also reactive to CD14 and CD64 by FC and to CD68 by immunohistochemical staining. The megakaryoblasts can be identified by CD41 and CD61, and the erythroblasts can be identified by glycophorin A and hemoglobin A staining.

Comparison of Flow Cytometry and Immunohistochemistry

Both FC and immunohistochemistry are not very useful in the initial diagnosis of CML because the immunophenotypes between CML and leukemoid reaction are very similar if the blast count is not high in the CML case. The time-honored test of leukocyte alkaline phosphatase (LAP) score, in contrast, is very useful as a screening technique in distinguishing these two entities. The cytoplasmic granules in the granulocytes of CML cases have low LAP activities; thus, the score is lower than the normal granulocytes in leukemoid reaction. However, the LAP activity is inhibited by anticoagulants; therefore, the blood for the test has

to be obtained by finger stick and a smear is to be made immediately. This is one of the reasons that the LAP score is not frequently performed. Naturally, it is more convenient for the clinician to order the sophisticated molecular genetic tests (e.g., FISH) for a prompt diagnosis, and the screening test is usually skipped.

Molecular Genetics

CML is characterized by the presence of t(9;22)(q34;q11.2) as detected by conventional karyotyping in 95% of patients. The shortened chromosome 22 was originally called the Ph chromosome, a term that is still used today. This genotype has now been verified by molecular biology as the translocation of a protooncogene, ABL1, on chromosome 9 to juxtapose the BCR gene on chromosome 21. As a result, a BCR-ABL1 fusion transcript is formed, and its product (a fusion BCR-ABL1 protein) acts as a constitutively active cytoplasmic tyrosine kinase. Because the breakpoint in the BCR gene can be at the minor BCR (m-bcr), major BCR (M-bcr), or micro BCR (mbcr), the fusion proteins are sized at 190, 210, and 230 kd, respectively.

All typical CML cases express a 210-kd BCR-ABL1 (P210). A subgroup of CML expressed a larger 230-kd BCR-ABL1 (P230) fusion protein and showed clinically a lower white cell count, prominent neutrophilic maturation, thrombocytosis, and slower progression than the typical CML (13). This subgroup is called neutrophilic CML to distinguish from chronic neutrophilic leukemia, which is Ph negative (14,15). Ph-positive acute lymphoblastic leukemia (ALL) cases express either a P210 or a P190 protein. In childhood ALL, 80% of patients carry the P190 protein (15). Adult CML with P190 protein is associated with monocytosis, mimicking chronic myelomonocytic leukemia (8). Rare cases of acute myeloid leukemia may be Ph positive, which may carry either P190 or P210 protein (16).



The role that the BCR-ABL1 protein plays in CML leukemogenesis is complicated. For this aspect, the reader is referred to several excellent review articles (1–3,17–20). In this section, only several well-established theories are briefly summarized. First, BCR-ABL1 protein can transform hematopoietic cells in vitro so that their growth and survival become independent of cytokines. The mechanism is through its tyrosine kinase activity to phosphorylate the tyrosine residues of several substrates (1,3,21). As a result, multiple signal transduction cascades affecting cell growth, differentiation, adhesion, and death are activated. These cells then escape normal constraints on growth and become leukemic. Second, BCR-ABL1 protein can protect hematopoietic cells from programmed cell death (apoptosis) in response to cytokine withdrawal and DNA damage (1–4,17–20,22). This effect is dependent on tyrosine kinase activity of the BCR-ABL1 protein and is reported to be associated with the activation of the Ras gene (17). As a result, these cells become immortal. In addition, because apoptosis does not occur after DNA damage in CML cells, the accumulated mutations in CML cells may finally lead to blast crisis (17,22). Third, defective adherence of immature hematopoietic CML cells to marrow stroma cells and extracellular matrix may facilitate their release into the blood and home to extramedullary locations or trap inside the blood compartment (1,17,18).

At the time of transformation to the AP and BP, cytogenetic evolution occurs in 50% to 80% of patients (2,5). The most common change is trisomy 8. In myeloblast crisis, double Ph chromosome, trisomy 8, trisomy 19, or isochromosome i(17q) may occur (7). The association between trisomy 8 and c-MYC overexpression and between isochromosome i(17q) and p53 mutation was suspected but has not been established (22). In contrast, the pathologic effects are more clear in t(3;21)(q26;q22) associated with expression of the AML-1/EVI-1 fusion protein and t(7;11)(9p15;p15) associated with expression of the NUP98/HOXA9 fusion protein (22). In addition, deletion or inactivation of tumor suppression genes, such as TP53, RB1, and p16, has also been associated with blast crisis in CML (2,18). As TP53 is genetically or functionally inactivated in a large fraction of CML cases in BP, it obviously plays an important role in CML blast crisis (2,22). Another tumor suppressor gene PP2A is also inhibited by BCR-ABL1 and is a candidate gene that leads to disease progression (23).

One of the most obvious differences between the normal ABL1 protein and the BCR-ABL1 fusion protein is their subcellular locations (1). The ABL1 protein is located in both the nucleus and cytoplasm, but the BCR-ABL1 fusion protein is located exclusively in the cytoplasm. In contrast, the vast majority of secondary changes involve genes encoding nucleus-localized proteins that regulate gene transcription (22). It is interesting that when imatinib inhibits the BCR-ABL1 tyrosine kinase in vitro, the BCR-ABL1 protein may enter into the nucleus of the culture cells (1). Therefore, it appears that the aberration of the genes that encode the nucleus-localized proteins, the subcellular location of BCR-ABL1 protein, and the progression of the disease are related sequences.

TABLE 6.1.2

Salient Features for Laboratory Diagnosis of CML

1. Leukocytosis: 50,000 to 100,000/ μ L
2. Wide spectrum of myeloid cells with myelocyte bulge, basophilia, and eosinophilia in the peripheral blood
3. Hypercellular bone marrow with particular increase in myelocytes or promyelocytes, basophilia, and eosinophilia
4. Blast count: CP < 5%; AP 10% to 19%; BP > 20%
5. Immunophenotype: positive for CD13, CD15, and CD33, and increased CD34 and CD117 proportional to blast counts
6. Cell lineage of blasts should be determined by FC or immunohistochemistry.
7. Low LAP score
8. Philadelphia chromosome, t(9;22), demonstrated by karyotyping
9. BCR-ABL1 gene/messenger RNA/protein detected by molecular biology techniques

The current case showed leukocytosis with immature myeloid cells and basophilia in the peripheral blood that led to the suspicion of CML. The high M/E ratio and disproportional high percentage of myelocytes in the peripheral blood and bone marrow also supported the diagnosis. Finally, it was the identification of BCR-ABL1 fusion product by FISH that established a diagnosis of CML. Although the total leukocyte count below 50,000/mL and the absence of splenomegaly are in favor of leukemoid reaction, the presence of many immature myeloid cells, particularly myeloblasts, in the peripheral blood makes leukemoid reaction the unlikely diagnosis. The FISH result is decisive in excluding this entity. If BCR-ABL1 fusion product is not detected, myelodysplastic/myeloproliferative neoplasms should be considered. The atypical chronic myeloid leukemia may have similar morphology in the peripheral blood and bone marrow, but the bone marrow should reveal prominent myeloid dysplasia. Chronic myelomonocytic leukemia may also show similar features, but there should be high monocyte counts in both peripheral blood and bone marrow with marked myelodysplastic changes.

The salient features for laboratory diagnosis of CML are summarized in Table 6.1.2.

Clinical Manifestations

CML is usually seen in patients between 40 and 60 years old, with a median age of 53 years (6,17). The male/female ratio is about 1.4:1. About 40% of patients are asymptomatic, and 50% of patients are diagnosed by routine testing (3). Therefore, most (85%) cases are diagnosed in the CP. Some patients may have a history of radiation exposure or previous chemotherapy, but the cause of CML in most patients is unknown.

TABLE 6.1.3

Comparison between CML and Leukemoid Reaction

	CML	Leukemoid reaction
Leukocyte count	Near 100,000/ μ L	Below 50,000/ μ L
% of promyelocytes/myelocytes	Higher	Lower
Blasts	Present	Absent
Basophilia	Present	Absent
Cytotoxic granules	Absent	Present
Myeloid/erythroid ratio	Near 10:1	Below 10:1
LAP score	Low	High
Splenomegaly	Frequently present	Usually absent
Philadelphia chromosome	Present	Absent
BCR-ABL1 fusion product	Present	Absent

The CP is manifested by an indolent clinical course. The symptoms are usually nonspecific, including fatigue, malaise, headache, weight loss, and anorexia. About 50% of patients have splenomegaly caused by extramedullary hematopoiesis (3,15). The high leukocyte number may cause leukostasis. If the patient has a high basophil count, he or she may have flushing secondary to hyperhistaminemia (17). However, the initial diagnosis is usually not based on clinical symptoms, but is due to a high leukocyte count with a wide spectrum of immature myeloid cells and increased numbers of basophils and eosinophils.

The CP may persist for 3 to 5 years and progress to accelerated and blastic phases (2). The AP may last for 1 year (21). When the patient reaches the BP, the median survival is about 18 weeks. The life expectancy of CML patients is about 4 years. However, about 10% of patients may survive for more than 8 years.

A form of atypical CML has been identified (6,7,24,25). Patients with this form have granulocytosis, sometimes monocytosis, but no Ph and BCR-ABL1 rearrangement. However, nonrecurrent cytogenetic abnormalities are present in most cases. Its characteristic laboratory findings include marked myeloid dysplasia and erythroid hypoplasia in the bone marrow. Its prognosis is significantly worse than CML. Therefore, atypical CML is considered a separate entity and is classified under myelodysplastic/myeloproliferative neoplasms in the WHO classification (5).

Pediatric patients may have the adult form of CML or juvenile myelomonocytic leukemia (JMML). JMML is Ph negative, but some cases may show other nonspecific cytogenetic abnormalities (6,26,27). These patients have leukocytosis, monocytosis, thrombocytopenia, and hepatosplenomegaly. The distinguishing features of JMML include frequent skin infiltration and elevation of fetal hemoglobin. The in vitro colony-forming assays, showing abundant spontaneous colony growth, 95% inhibition of colony by antibodies to granulocyte-macrophage colony-stimulating factor, and hypersensitivity to granulocyte-macrophage colony-stimulating factor, are relatively specific for the diagnosis of JMML. JMML is classified under

myelodysplastic/myeloproliferative neoplasms in the WHO classification (5).

The adult form of chronic myelomonocytic leukemia is similar to JMML in clinical and laboratory aspects (4,28). The reader is referred to Case 4 for a detailed discussion.

As mentioned before, the initial diagnosis may be based on the examination of the peripheral blood. The LAP score may help to distinguish the CP of CML from a leukemoid reaction. However, in the AP and BP, the LAP score may become gradually increased. Thus, these two entities should be distinguished on multiple morphologic parameters (Table 6.1.3) (6,7,29). A definitive diagnosis, however, depends on the identification of karyotype by conventional cytogenetics or BCR-ABL1 fusion product by molecular cytogenetic techniques (17,18,30).

The Southern blot analysis is able to identify the rearranged BCR gene (Fig. 6.1.11). The Western blot analysis can detect the BCR-ABL1 protein. The FISH technique can detect the fusion gene in interphase and/or metaphase nuclei (Fig. 6.1.12) (31). This technique can be applied to dried smears of peripheral blood and bone marrow, and it is the method of choice for initial diagnosis as it can identify the fusion product in 100% of CML cases (5). The conventional karyotyping can only identify 95% of CML cases because of the existence of cryptic translocation (1,5,19). However, it is able to demonstrate additional cytogenetic aberrations and is thus particularly useful in identifying patients transforming from CP to AP or BP (5).

The most sensitive technique is the reverse transcriptase-polymerase chain reaction (RT-PCR), which is used to detect BCR-ABL1 messenger RNA transcript. The nested RT-PCR is even more sensitive than RT-PCR (25). However, the qualitative RT-PCR techniques are labor intensive and difficult to standardize. Therefore, they have been gradually replaced by the quantitative real-time PCR techniques, using either the TaqMan or LightCycle system (32,33).

For therapeutic monitoring, it is recommended that karyotyping or FISH be used in the early phase of treatment. When Ph is no longer detectable, serial quantitative RT-PCR studies should be performed at approximately

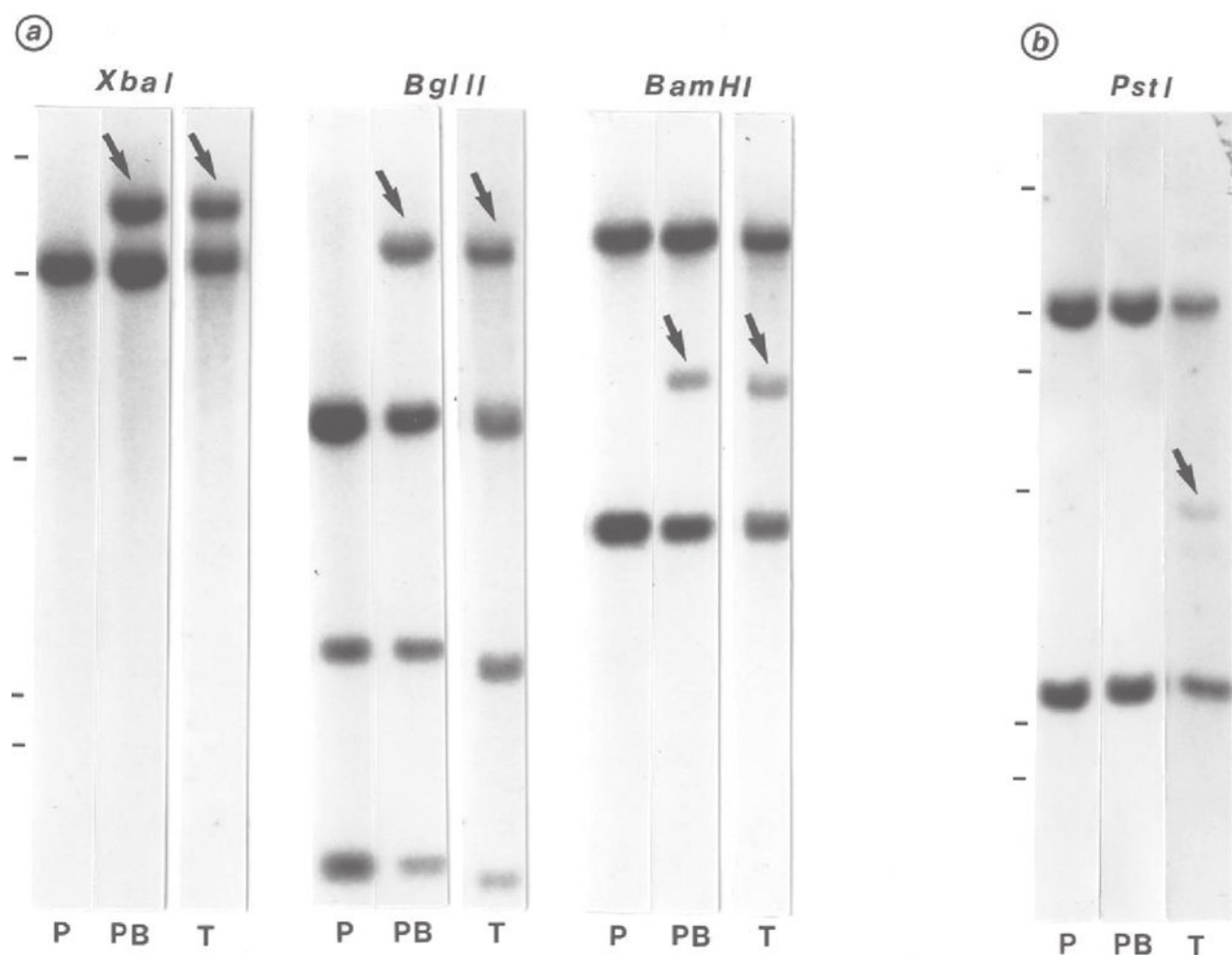


FIGURE 6.1.11 **A:** Southern blot hybridization analysis of DNA from peripheral blood (PB) and extramedullary tumor tissue (T) with transprobe-1 (for BCR or Philadelphia chromosome) showing identical rearrangement bands (arrows) after digestion with *Xba*I, *Bgl*II, and *Bam*HI. P, placental control. **B:** Same tissues reacted with T-cell receptor b chain probe showing a faint rearranged band (arrow) in tumor tissue (T) after *Pst*I digestion. (From Sun T, Susin M, Koduru P, et al. Extramedullary blast crisis in chronic myelogenous leukemia. *Cancer*. 1991;68:605–610, with permission.)

3-month intervals (33). The current standard of therapeutic monitoring is based on three different types of responses (9,16). The complete hematologic response is defined by a platelet count of $<450 \times 10^9/L$, no immature granulocyte and $<5\%$ basophils in peripheral blood, and nonpalpable spleen. The peripheral blood should be checked every two weeks until complete response is achieved and confirmed. Then hematologic studies should be done every 3 months.

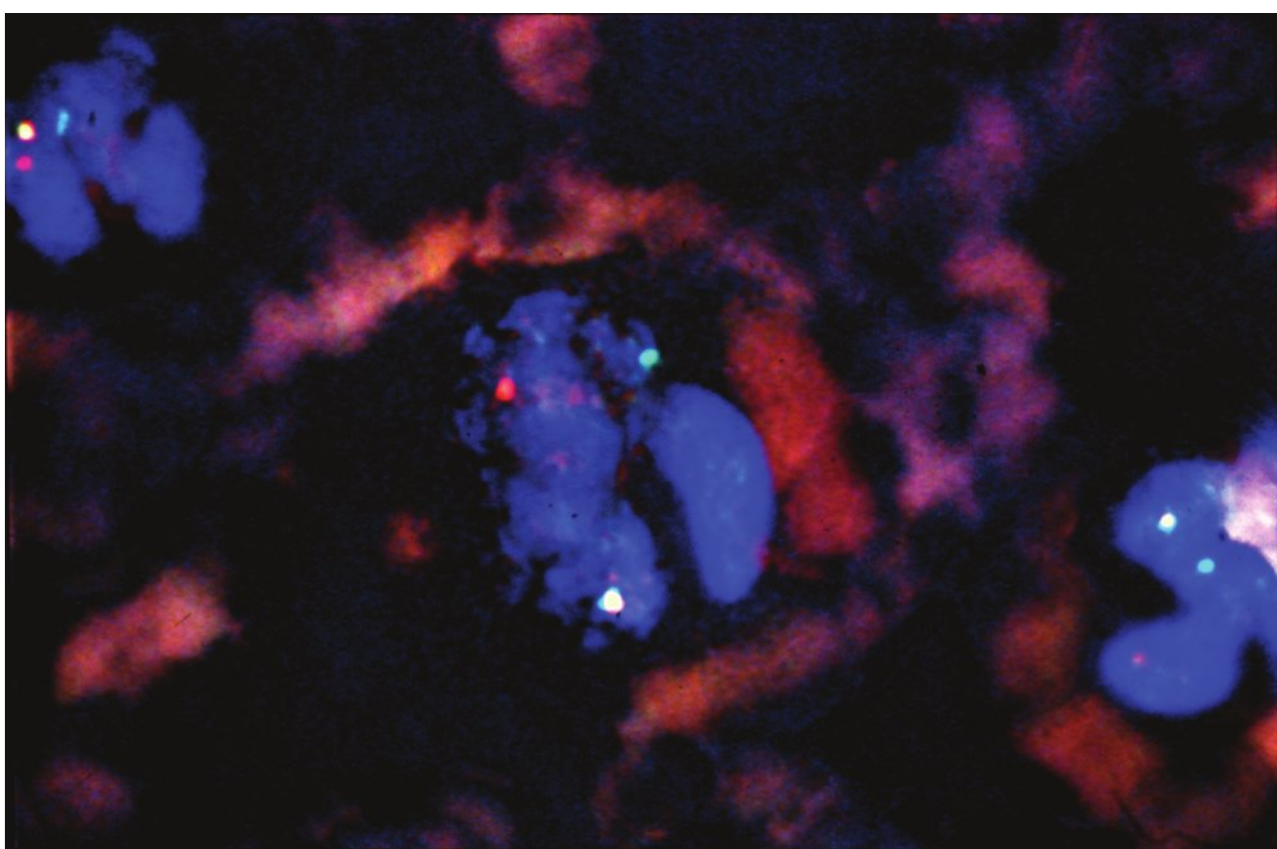


FIGURE 6.1.12 FISH of peripheral blood from a CML patient reacting with BCR and Ableson 1 (ABL1) probes, showing a green signal (BCR), a red signal (ABL1), and a yellow signal, which represents the overlapping of a green and red signal, in all three segmented neutrophils, indicative of BCR-ABL1 gene fusion product.

A complete cytogenetic response is defined by absence of Ph-positive cells in the peripheral blood or bone marrow. A partial cytogenetic response is defined as presence of 1% to 35% Ph-positive cells. Cytogenetic studies of bone marrow should be done at least every 6 months until complete response achieved and confirmed. Then cytogenetic studies should be done at least every 12 months.

A complete molecular response is indicated by non-quantifiable and nondetectable BCR-ABL1 transcript. A major molecular response is defined by a reduction of BCR-ABL1 transcript by 3 or more logs below a standard baseline value. The NIH introduces the International Scale, in which the baseline value is designated 100% and the 3-log reduction becomes 0.1%. The achievement of a major molecular response is correlated with progression-free survival. In other words, the patients remain in CP and do not progress to AP and BP, resulting in a long survival. Molecular monitoring should be performed every 3 months in follow-up.

During CP, cytoreductive therapy is needed to prevent leukostasis. Hydrourea and busulfan may induce hematologic but not cytogenetic remission (2,3,17). High-dose chemotherapy followed by allogeneic bone marrow transplantation used to be the only means to achieve cytogenetic cure. In patients who are not suitable candidates for bone marrow transplant, the alternative therapy was the use of interferon alfa, which can induce both hematologic and cytogenetic remission in CP. However, the recent use of BCR-ABL1 tyrosine kinase inhibitors has revolutionized the treatment of CML (18,34). The most commonly used drug is imatinib mesylate with the trade names of Gleevec in the United States and Glivec in Europe. It competitively binds to the adenosine triphosphate-binding site of the BCR-ABL1 and inhibits protein tyrosine phosphorylation (35). Imatinib mesylate induces complete cytogenetic responses in up to 90% of patients and major molecular responses in most of them (34).

However, the emergence of subclones of leukemic progenitor cells with point mutation may prevent the binding of the tyrosine kinase inhibitors to the kinase domain of BCR-ABL1, thus leading to drug resistance to imatinib (16,36,37). The second generation of inhibitors, such as nilotinib and dasatinib, can circumvent this mechanism and are effective in treating most of the imatinib-resistant cases (36,37). Dasatinib may also stimulate the proliferation of clonal natural killer cells/cytotoxic T cells, leading to a favorable prognosis (38). It is hypothesized that dasatinib may restore the function of anergic, exhausted leukemia-specific cytotoxic lymphocytes by inhibiting distinct off target kinases in immune effector cells (38).

REFERENCES

1. Goldman JM, Melo JV. Chronic myeloid leukemia—advances in biology and new approaches to treatment. *N Engl J Med*. 2003;349:1451–1464.
2. Faderl S, Talpaz M, Estrov Z, et al. The biology of chronic myeloid leukemia. *N Engl J Med*. 1999;341:164–172.
3. Sawyers C. Chronic myeloid leukemia. *N Engl J Med*. 1999;340:1330–1340.

4. Anastasi J, Vardiman JW. Chronic myelogenous leukemia and the chronic myeloproliferative diseases. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1745–1790.
5. Vardiman JW, Pierre R, Thiele J, et al. Chronic myelogenous leukemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:20–26.
6. Bruning RD, McKenna RW. *Tumor of the Bone Marrow*. Washington, DC: Armed Forces Institute of Pathology; 1994:195–299.
7. Foucar K. *Bone Marrow Pathology*. Chicago, IL: ASCP Press; 2001:204–213.
8. Vardiman JW, Melo JV, Baccarani M, et al. Chronic myelogenous leukaemia, BCR-ABL 1 positive. In: Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:32–37.
9. Baccarani M, Saglio G, Goldman J, et al. Evolving concepts in the management of chronic myeloid leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. *Blood* 2006;108:1809–1820.
10. Sun T, Susin M, Koduru P, et al. Extramedullary blast crisis in chronic myelogenous leukemia. *Cancer*. 1991;68:605–610.
11. Banavali S, Silvestri F, Hulette B, et al. Expression of hematopoietic progenitor cell associated antigen CD34 in chronic myeloid leukemia. *Leuk Res*. 1991;15:603–608.
12. Orazi A, Neiman RS, Cualing H, et al. CD34 immunostaining of bone marrow biopsy specimens is a reliable way to classify the phases of chronic myeloid leukemia. *Am J Clin Pathol*. 1994;101:426–428.
13. Pane F, Frigeri F, Sindona M, et al. Neutrophilic-chronic myeloid leukemia: a distinct disease with a specific molecular marker. *Blood*. 1996;88:2410–2414.
14. Reilly JT. Chronic neutrophilic leukaemia: a distinct clinical entity? *Br J Haematol*. 2002;116:10–18.
15. Mauro MJ, Druker BJ. Chronic myelogenous leukemia. *Curr Opin Oncol*. 2001;13:3–7.
16. Ross DM, Hughes TP. Current and emerging tests for the laboratory monitoring of chronic myeloid leukaemia and related disorders. *Pathology*. 2008;40:231–246.
17. Thijsen SFT, Schuurhuis GJ, van Oostveen JW, et al. Chronic myeloid leukemia from basic to bedside. *Leukemia*. 1999;13:1646–1674.
18. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. *Blood*. 2000;96:3343–3356.
19. Kurzrock R, Kantarjian HM, Druker BJ, et al. Philadelphia chromosome-positive leukemias: from basic mechanism to molecular therapeutics. *Ann Intern Med*. 2003;138:819–830.
20. Holyoak TL. Recent advances in the molecular and cellular biology of chronic myeloid leukaemia: lessons to be learned from the laboratory. *Br J Haematol*. 2001;113:11–23.
21. Kulidas M, Kantarjian H, Talpaz M. Chronic myelogenous leukemia. *JAMA*. 2001;286:895–898.
22. Calabretta B, Perrotti D. The biology of CML blast crisis. *Blood*. 2004;103:4010–4022.
23. Nevianai P, Santhanam R, Trotta R, et al. The tumor suppressor PP2A is functionally inactivated in blast crisis CML through the inhibitory activity of the BCR/ABL-regulated SET protein. *Cancer Cell* 2005;8:355–368.
24. Hernandez JM, del Canizo MC, Cuneo A, et al. Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. *Ann Oncol*. 2000;11:441–444.
25. Oscier D. Atypical chronic myeloid leukemias. *Pathol Biol*. 1997;45:587–593.
26. Hess JL, Zutter MM, Castleberry RP, et al. Juvenile chronic myelogenous leukemia. *Am J Clin Pathol*. 1996;105:238–248.
27. Chomienne C, Cambier N, Baruchel A. Juvenile chronic myelogenous leukemias: molecular and novel therapeutic basis. *Pathol Biol*. 1997;45:600–604.
28. Orazi A, Bennett JM, Germing U, et al. Chronic myelomonocytic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008:76–79.
29. Naeim F. *Atlas of Bone Marrow and Blood Pathology*. Philadelphia, PA: W. B. Saunders; 2001:53–57.
30. Hochhause A, Weisser A, La Rosee P, et al. Detection and quantification of residual disease in chronic myelogenous leukemia. *Leukemia*. 2000;14:998–1005.
31. Sinclair PB, Green AR, Grave C, et al. Improved sensitivity of BCR-ABL detection: a triple-probe three-color fluorescence in situ hybridization system. *Blood*. 1997;90:1395–1402.
32. Elmaagacli AH, Beelen DW, Opalka B, et al. The amount of BCR-ABL fusion transcripts detected by the real-time quantitative polymerase chain reaction method in patients with Philadelphia chromosome positive chronic myeloid leukemia correlated with the disease stage. *Ann Hematol*. 2000;79:424–431.
33. Goldman J. Monitoring minimal residual disease in BCR-ABL-positive chronic myeloid leukemia in the imatinib era. *Curr Opin Hematol*. 2004;12:33–39.
34. Cortes J, Kantarjian H. New targeted approaches in chronic myeloid leukemia. *J Clin Oncol*. 2005;23:6316–6324.
35. Tsao AS, Kantarjian H, Talpaz M. STI-571 in chronic myelogenous leukaemia. *Br J Haematol*. 2002;119:15–24.
36. Kantarjian HM, Talpaz M, Giles F, et al. New insights into the pathophysiology of chronic myeloid leukemia and imatinib resistance. *Ann Intern*. 2006;145:913–923.
37. Weisberg E, Manley PW, Cowan-Jacob SW, et al. Second generation inhibitors of BCR-ABL for the treatment of imatinib-resistant chronic myeloid leukaemia. *Nat Rev Cancer* 2007;7:345–356.
38. Kreutzman A, Juvonen V, Kairisto V, et al. Mono/oligoclonal T- and NK-cells are common in chronic myeloid leukemia patients at diagnosis and expand during dasatinib therapy. *Blood* 2010;116:772–782.

CASE 2

BCR-ABL1–Negative Myeloproliferative Neoplasms

CASE HISTORY

A 71-year-old man was admitted for splenectomy. He had a 5-year history of splenomegaly with abdominal discomfort and right upper quadrant pain. Review of past history showed that he was diagnosed polycythemia vera (PV) 5 years ago and had been treated with Anagrelide and hydroxyurea in subsequent years. He was also found to have large ascites by ultrasound before admission. Physical examination showed a massive spleen extending to the iliac crest, fluid wave in the abdomen, and pitting edema in the lower extremities. Laboratory studies revealed a total leukocyte count of 16,500/mL, hemoglobin 11.8 g/dL, hematocrit 35%, platelet count 325,000/mL, and lactate dehydrogenase 690 U/L. No immature myelomonocytic cells or nucleated erythrocytes were demonstrated in the peripheral blood.

During the operation of splenectomy, his liver was found to have macronodular cirrhosis and five liters of ascites were removed. The splenectomy specimen showed extramedullary hematopoiesis (Fig. 6.2.1). A bone marrow biopsy confirmed myelofibrosis with megakaryocytic hyperplasia (Fig. 6.2.2). About 1 month later, the patient had left upper quadrant pain, and a CT scan of the abdomen and pelvis revealed a portal vein thrombosis. He was then placed on intravenous heparin, which was ultimately converted to Coumadin therapy. Throughout his hospital course, the patient was treated with Anagrelide and hydroxyurea and was monitored with leukocyte and platelet counts. Because of several episodes of cytopenia, the need of parenteral nutrition and wound infections, the patient stayed in the hospital for nearly 3 months and was eventually transferred to a nursing home. During his nursing home stay, his treatment was continually monitored by his oncologist from the hospital. He died of acute gastric hemorrhage 3 years later.

MOLECULAR GENETICS

Karyotyping on both peripheral blood and bone marrow specimens showed a normal male karyotype 46, XY.

Fluorescence in situ hybridization for breakpoint cluster region (BCR)-Ablson 1 (ABL1) fusion product on bone marrow was negative.

DISCUSSION

The concept of myeloproliferative disorders was first developed by William Dameshek in 1951, when

he described a group of disorders characterized by panmyeloid proliferations and suggested that this might result from an undiscovered stimulus (1,2). The original members included chronic myelogenous leukemia (CML), PV, essential thrombocythemia (ET), chronic idiopathic myelofibrosis (primary myelofibrosis [PMF]), and erythroleukemia (Di Guglielmo syndrome) (3). The discovery of Philadelphia chromosome/BCR-ABL1 fusion product in CML first separated CML from this group. Then, erythroleukemia has been redefined as acute erythroid leukemia. As a result, only four members remain in the group, and they are called classic or BCR-ABL–negative myeloproliferative disorders (4,5). The 2001 World Health Organization (WHO) classification of myeloid malignancies designates this group of disorders as chronic myeloproliferative diseases and includes chronic neutrophilic leukemia, chronic eosinophilic leukemia/hypereosinophilic syndrome, chronic myeloproliferative disease, unclassifiable in the same category (6). The 2008 WHO classification changes the designation to myeloproliferative neoplasms (MPNs) because of the association of Janus kinase 2 (JAK2), thrombopoietin receptor (MPL) mutations, and other genetic markers with these disorders (7). Mast cell disease is added to this new category and eosinophilia associated with PDGFRA, PDGFRB, and FGFR1 becomes a separate entity. The term BCR-ABL–negative MPNs is used in the discussion of this case.

Morphology

MPN is a group of disorders with overproduction of mature, functional blood cells and are considered to arise from a multipotent hematopoietic progenitor (8). Therefore, the clinical and pathologic features of these disorders are frequently overlapping, and a clear separation from one disorder to another is sometimes difficult. Because the neoplastic cells are mature with normal function, MPN is even difficult to distinguish from reactive myeloproliferative diseases, such as secondary polycythemia and reactive thrombocytosis. However, each disorder has its cardinal features: an increased red-cell mass in PV, a high platelet count in ET, and bone marrow fibrosis in PM. They also share some common features, such as marrow hypercellularity, propensity of thrombosis and hemorrhage, myelofibrosis in terminal stage, and potential transformation to acute leukemia (8). They can also transform into each other. The discovery of JAK2 mutation and other molecular genetic markers greatly facilitates the differential diagnosis between MPN and reactive myeloproliferative diseases, but it does not help the distinction of the three members of MPN as they share the same abnormalities.

The first organization that attempted to establish diagnostic criteria for the diagnosis of PV and ET was

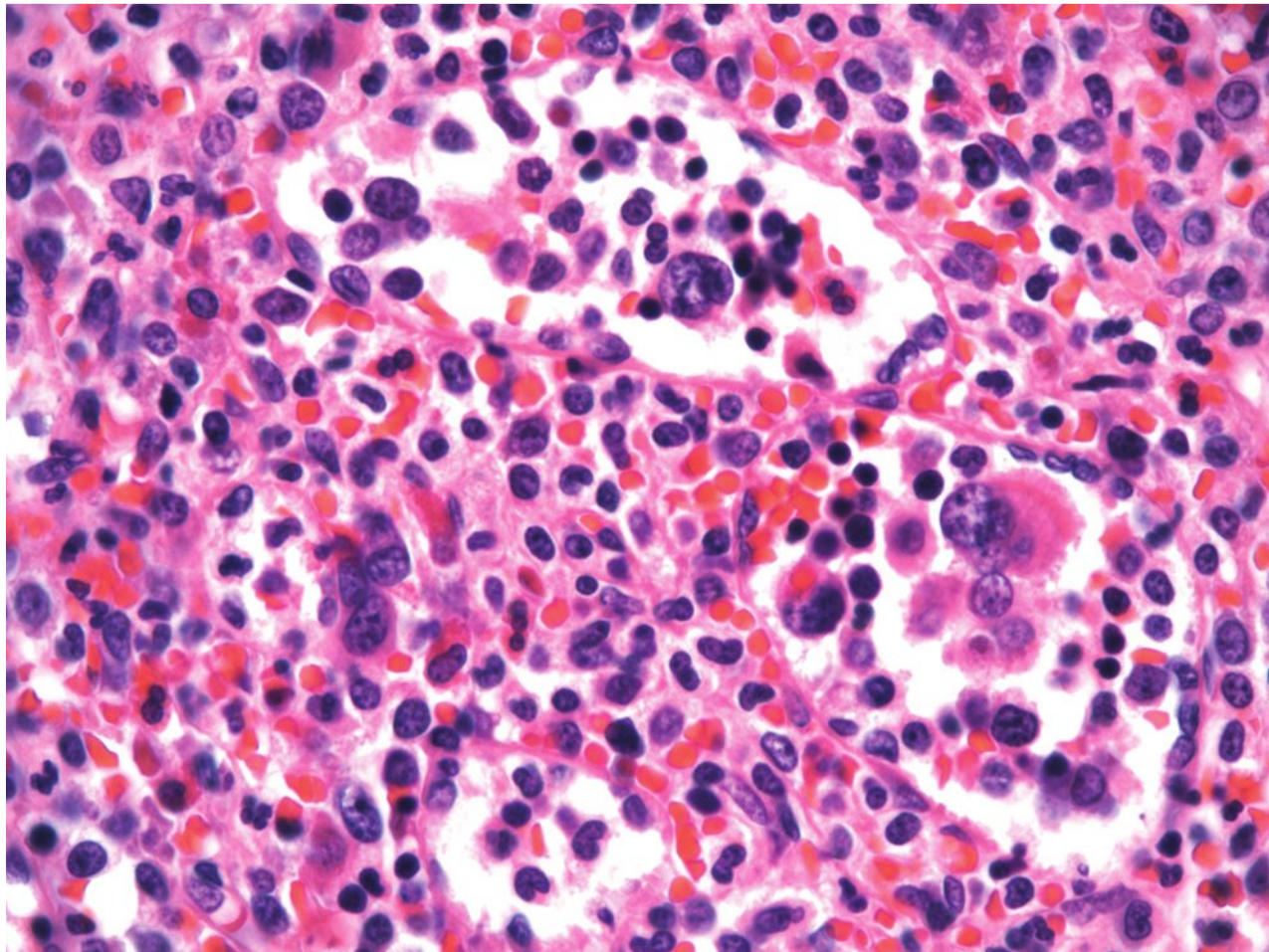


FIGURE 6.2.1 Splenectomy specimen shows megakaryocytes and nucleated erythrocytes in the red pulp sinuses and the cords of Billroth, representing extramedullary hematopoiesis. H&E, $\times 60$.

the Polycythemia Vera Study Group (PVSG) (9). Its criteria are mainly focused on the exclusion of other causes of erythrocytosis and thrombocytosis. Its major weakness was the suboptimal use of bone marrow histology, which was subsequently corrected (10). The PVSG guidelines emphasized the measurement of red cell mass and plasma volume for the diagnosis of PV (11). However, the identification of specific molecular markers in practically every case of PV makes this tedious procedure obsolete (3,12). The 2008 WHO classification emphasizes the constellation of clinical laboratory findings with morphologic features and molecular genetic data for the diagnosis of PMN (3,12). These guidelines have been generally accepted by clinicians and pathologists in spite of some objections (11).

The bone marrow morphology of PV can be divided into pre-PV, overt PV, and spent (post-PV myelofibrosis) phases (7). The pre-PV phase is similar to the overt PV

phase, except that it does not fulfill the diagnostic criteria of PV in terms of hematocrit, hemoglobin, or red cell mass (7,12–14). However, even in the polycythemic phase, the patient may have hypochromic, microcytic anemia because of the existence of hemorrhagic complications. Panmyelosis is the hallmark of both phases with quantitative differences. The peripheral blood may show erythrocytosis, granulocytosis, and/or thrombocytosis. The bone marrow is usually hypercellular with panmyelosis, in which megakaryocytosis is most characteristic for differential diagnosis (Fig. 6.2.3). The megakaryocytes usually form clusters and are pleomorphic, varying from small to giant size, but no prominent morphologic abnormalities are demonstrated in most cases (Fig. 6.2.4). Atypical forms are rarely seen. If atypia or dysplasia is a prominent feature, other conditions should be considered. Erythropoiesis is normoblastic and granulopoiesis is normal morphologically.

In secondary polycythemia, the marrow may show various degrees of proliferation in granulocytes and erythrocytes, depending on the etiology, but megakaryocytic hyperplasia is usually absent. On the other hand, when megakaryocytic hyperplasia is prominent in pre-PV cases, it may mimic ET. However, the megakaryocytes in ET are usually large with hyperlobulation. The stromal reaction pattern in PV, such as perivascular plasmacytosis, cell debris-laden macrophages, and mature eosinophils, is also not seen in ET (15). Another special feature in PV is increased cellularity in subcortical marrow space, where it is normally hypocellular (16). Iron stain is helpful in distinguishing PV from other MPN entities, because 95% of PV cases showed absence of iron stores due to excessive utility of iron for erythropoiesis. There are many other parameters that may help to distinguish PV from secondary or congenital erythrocytosis; the reader is referred to the review articles by Patnaik and Tefferi (17) and Zhan and Spivak (18).

The post-PV phase is characterized by a progressive decrease of red cell mass, increase in marrow fibrosis and splenomegaly due to extramedullary hematopoiesis.

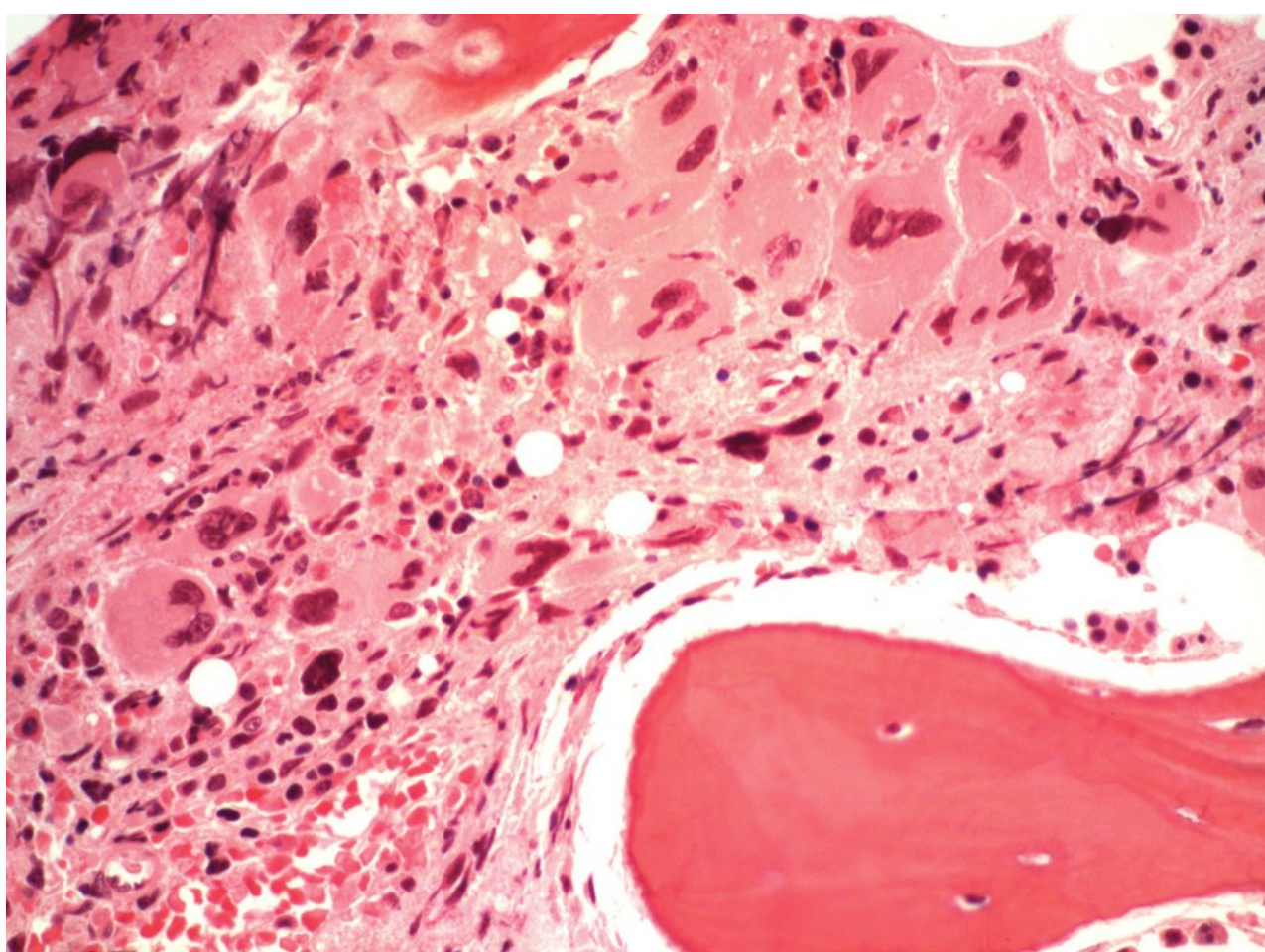


FIGURE 6.2.2 Bone marrow biopsy reveals myelofibrosis and megakaryocytic hyperplasia. H&E, $\times 40$.

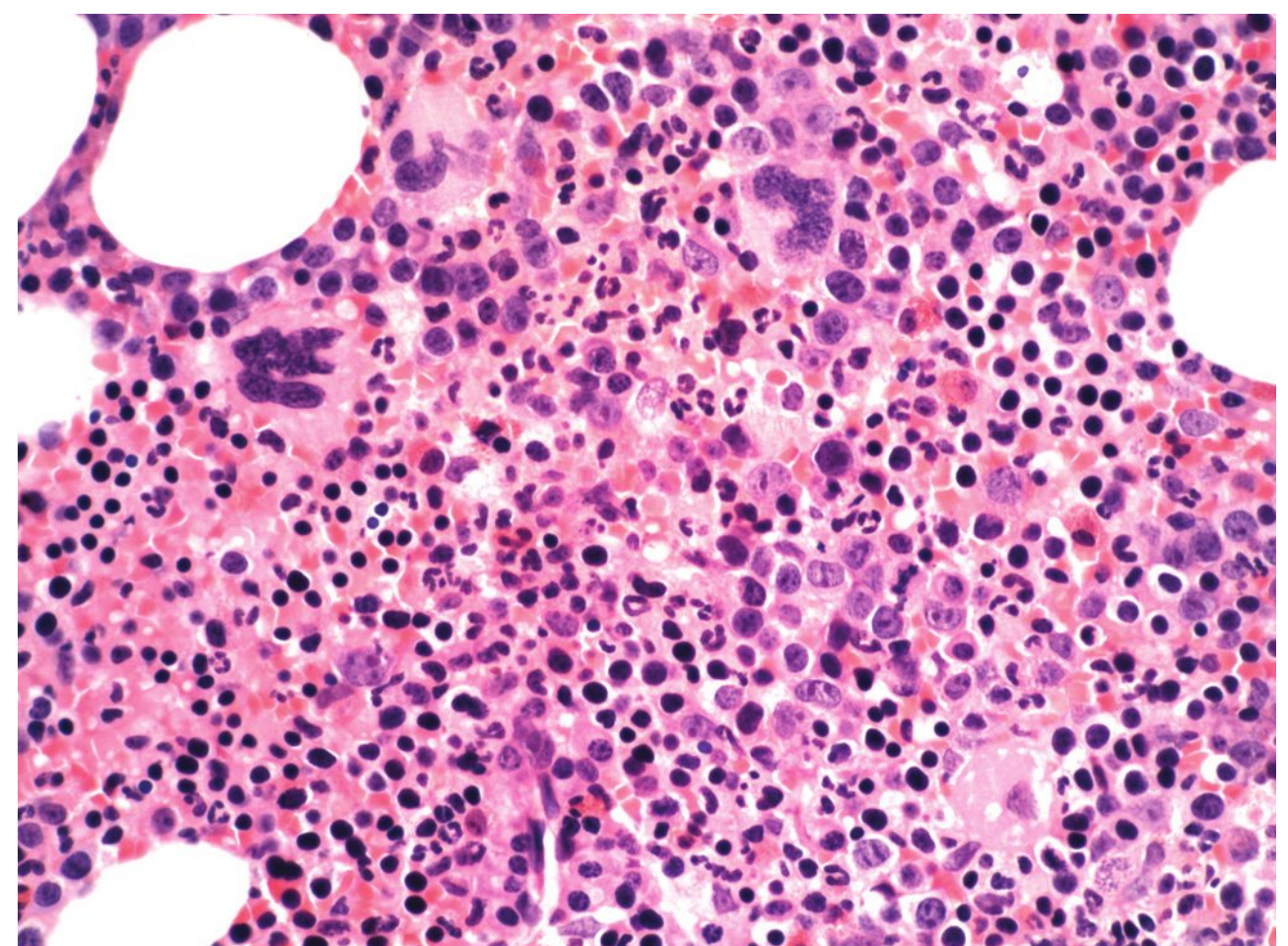


FIGURE 6.2.3 Bone marrow biopsy demonstrates panmyelosis with megakaryocytes in variable sizes. H&E, $\times 40$.

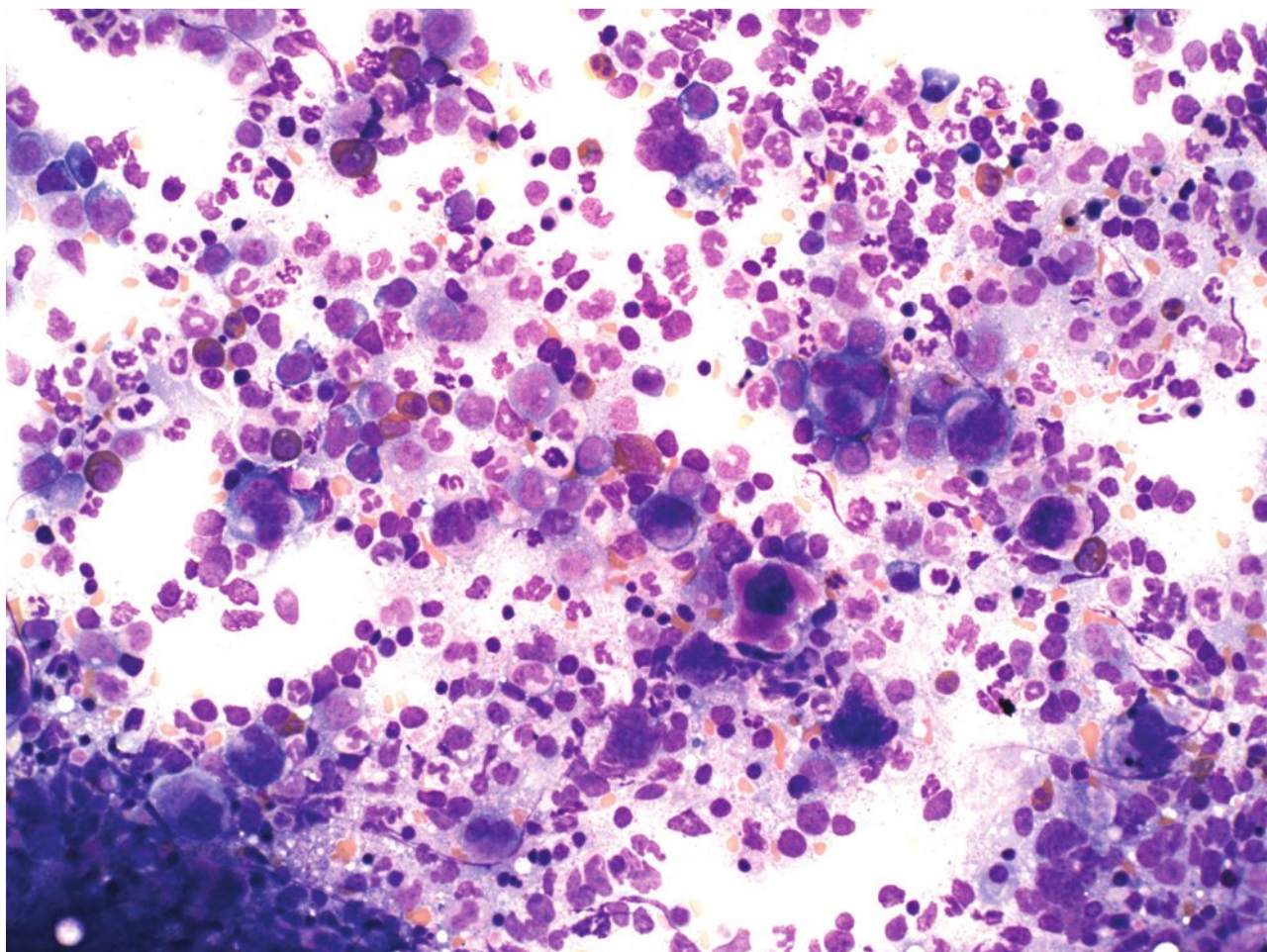


FIGURE 6.2.4 Bone marrow aspirate shows multiple megakaryocytes with proliferating normoblasts and myelocytic series in the background. Wright-Giemsa, ×20.

As a result, the red cell mass, hemoglobin, and hematocrit are normalized and gradually decreased to an anemic level. Similar to PMF, the peripheral blood may show leukoerythroblastosis (presence of nucleated red cells and immature myeloid cells), poikilocytosis with dacryocytes (teardrop-shaped red cells). The bone marrow shows decreased erythropoiesis and granulopoiesis, but megakaryocytic hyperplasia is still present. The megakaryocytes may show atypical forms with hyperchromatic nuclei similar to those seen in PMF. Immature myeloid cells may be found in bone marrow, but blasts are usually <10%. Reticular and collagen fibrosis gradually become prominent. As a result, the marrow sinuses are dilated and contain red cells, granulocytes, and megakaryocytes. Osteosclerosis may also occur. The splenectomy specimen also shows the trilineage hematopoietic cells in the splenic sinuses and cords of Billroth. All the above-mentioned features are so similar to those seen in PMF that sometimes it is difficult to distinguish these two conditions without a previous history of polycythemic phase.

ET is considered a diagnosis by exclusion by many authors because thrombocytosis is also a common phenomenon in other MPN entities, and there is no clear morphologic marker for its identity (11,12,19). Its major characteristic is megakaryocytic hyperplasia without obvious increase in erythropoiesis and granulopoiesis. The detection of JAK2 or other mutations may help to differentiate ET from reactive thrombocytosis, but it is the bone marrow examination that helps the distinction between different MPN entities. Because of the high threshold of platelet count required for the diagnosis of ET previously, many early cases were underdiagnosed. Those cases were called prodromal stages of ET. It is important to recognize these cases because severe vascular events such as thrombosis and hemorrhage may occur in this stage. However, the revised guidelines of the WHO reduce the diagnostic threshold of platelet count from 600,000 to 450,000/mL so that many “prodromal” cases may now be recognized as overt ET.

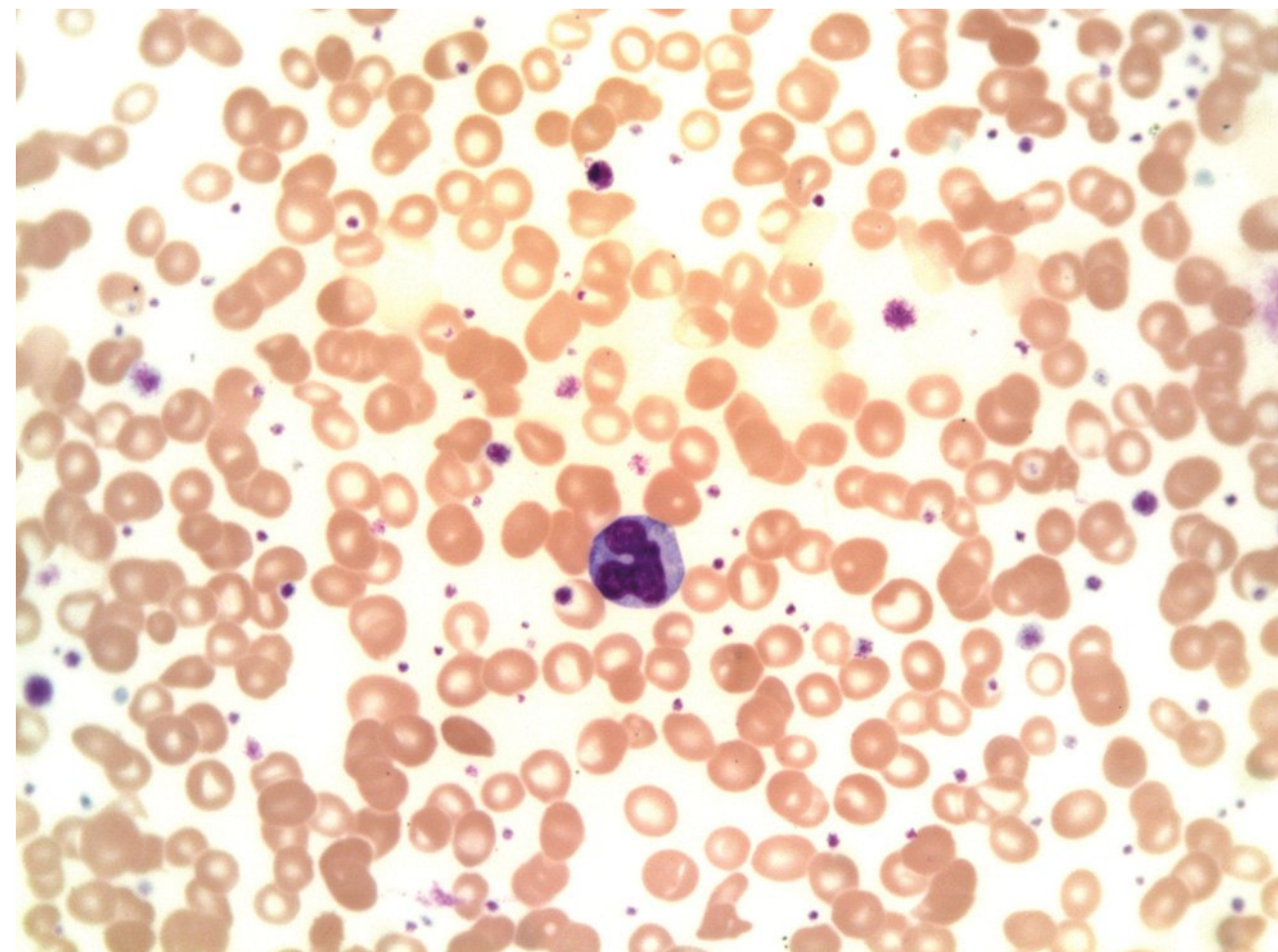


FIGURE 6.2.5 Peripheral blood smear reveals thrombocytosis with anisocytosis of the platelets. Wright-Giemsa, ×60.

In the peripheral blood of ET cases, the total leukocyte and differential counts are within normal ranges. The erythrocytes are normochromic and normocytic unless the patient has hemorrhagic episodes that may induce hypochromic, microcytic anemia. Leukoerythroblastosis is not seen in ET cases. By definition, platelet count is elevated with anisocytosis (coexistence of small to large, giant forms) (Fig. 6.2.5). Occasionally, bizarre forms, pseudopods, and agranular platelets may be present. In the bone marrow, there is no proliferation of erythroid and myeloid series, unless hemorrhages occur. Myelodysplasia or increased blasts are not seen. On the other hand, megakaryocytic hyperplasia is obvious. The megakaryocytes are large to giant form with hyperlobulation and abundant mature cytoplasm (Fig. 6.2.6). Highly atypical or dysplastic forms are not seen. Emperipoiesis (phagocytosis of blood elements by megakaryocytes) is frequently seen but it

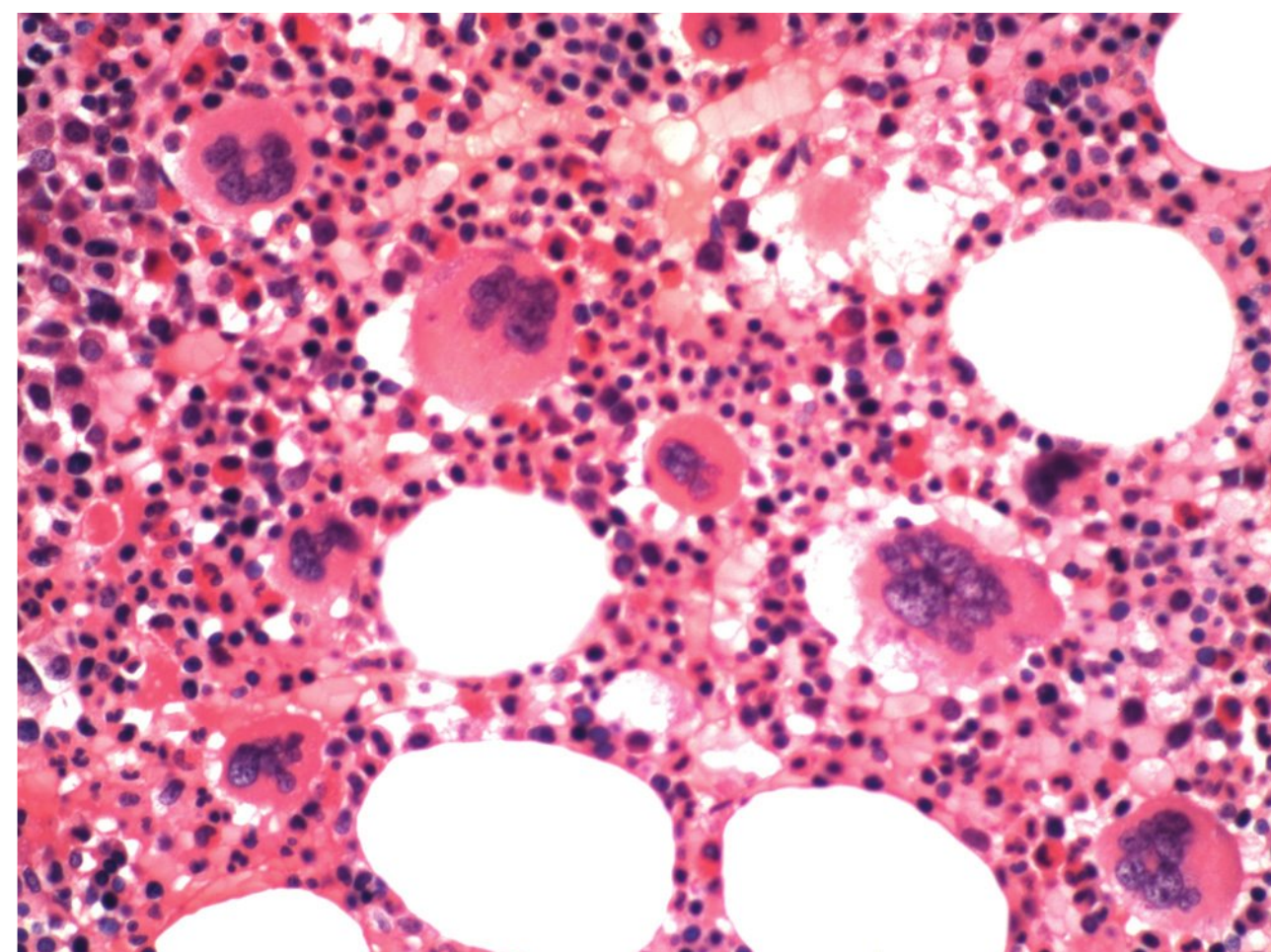


FIGURE 6.2.6 Bone marrow biopsy demonstrates a loose cluster of megakaryocytes, composed of large, hyperlobated cells, characteristic of ET. H&E, ×40.

is not a specific finding for ET. In contrast to the dense clustering seen in PMF and PV, the megakaryocytes are dispersed or form loose clusters. The bone marrow aspirates may demonstrate increase numbers of large, hyperlobated megakaryocytes with large sheets of platelets in the background. The reticulin network is normal or slightly increased. The iron stores are also normal in 40% to 70% of cases (19).

PMF is clinically indistinguishable from the transformation of PV or ET to myelofibrosis (8) and the attempt to make a definitive diagnosis is probably not significant because their treatment is similar. The early diagnostic criteria of PMF were adopted by the Italian consensus conference, which included two necessary criteria (diffuse bone marrow fibrosis and absence of Philadelphia chromosome or BCR-ABL rearrangement in peripheral blood) (20). In addition, there were six optional criteria: (a) splenomegaly of any grade, (b) anisopoikilocytosis with teardrop erythrocytes (Fig. 6.2.7), (c) presence of circulating immature myeloid cells, (d) presence of circulating erythroblasts, (e) presence of cluster of megakaryoblasts and anomalous megakaryocytes in bone marrow sections, and (f) myeloid metaplasia.

The original WHO classification of PMF was divided into prefibrotic and fibrotic phases. The diagnostic criteria include clinical findings and morphologic findings (6). The clinical presentations are splenomegaly and hepatomegaly; moderate-to-marked anemia; and normal, decreased, or increased leukocytes and platelets. Morphologic manifestations in the peripheral blood include leukoerythroblastosis (Fig. 6.2.8), prominent red blood cell poikilocytosis, and prominent dacryocytosis. The bone marrow criteria are composed of reticulin and/or collagen fibrosis, decreased cellularity, dilated marrow sinuses, intraluminal hematopoiesis, neutrophilic proliferation, prominent megakaryocytic proliferations, megakaryocytic atypia, and new bone formation (osteosclerosis). For prefibrotic PMF, the bone marrow fibrosis may be mild or absent with marked myelopoiesis (cellular phase). Compared to the fibrotic phase, the other clinical or morphologic changes are milder or absent.

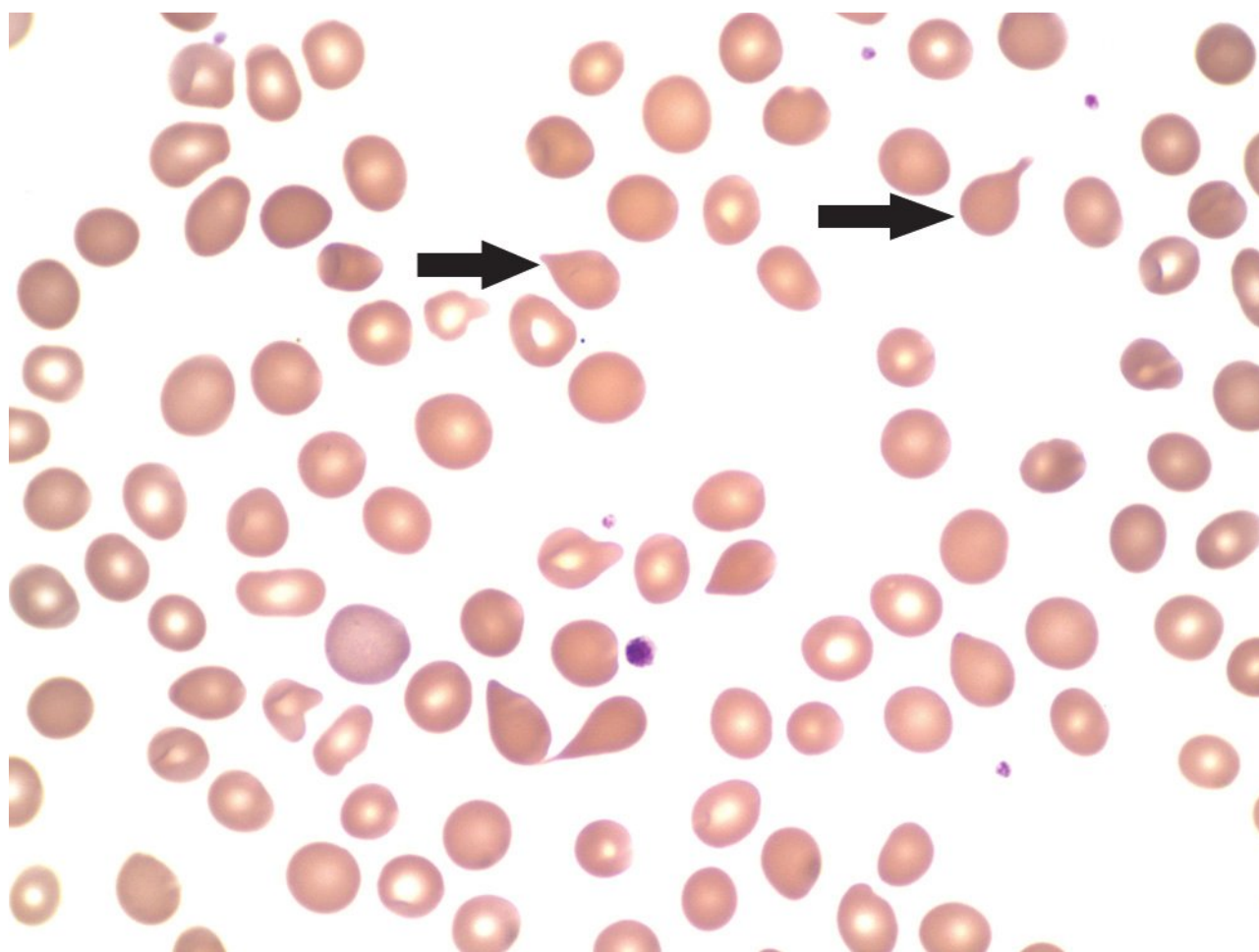


FIGURE 6.2.7 Peripheral blood smear shows several teardrop-shaped erythrocytes (arrows). Wright–Giemsa, $\times 100$.

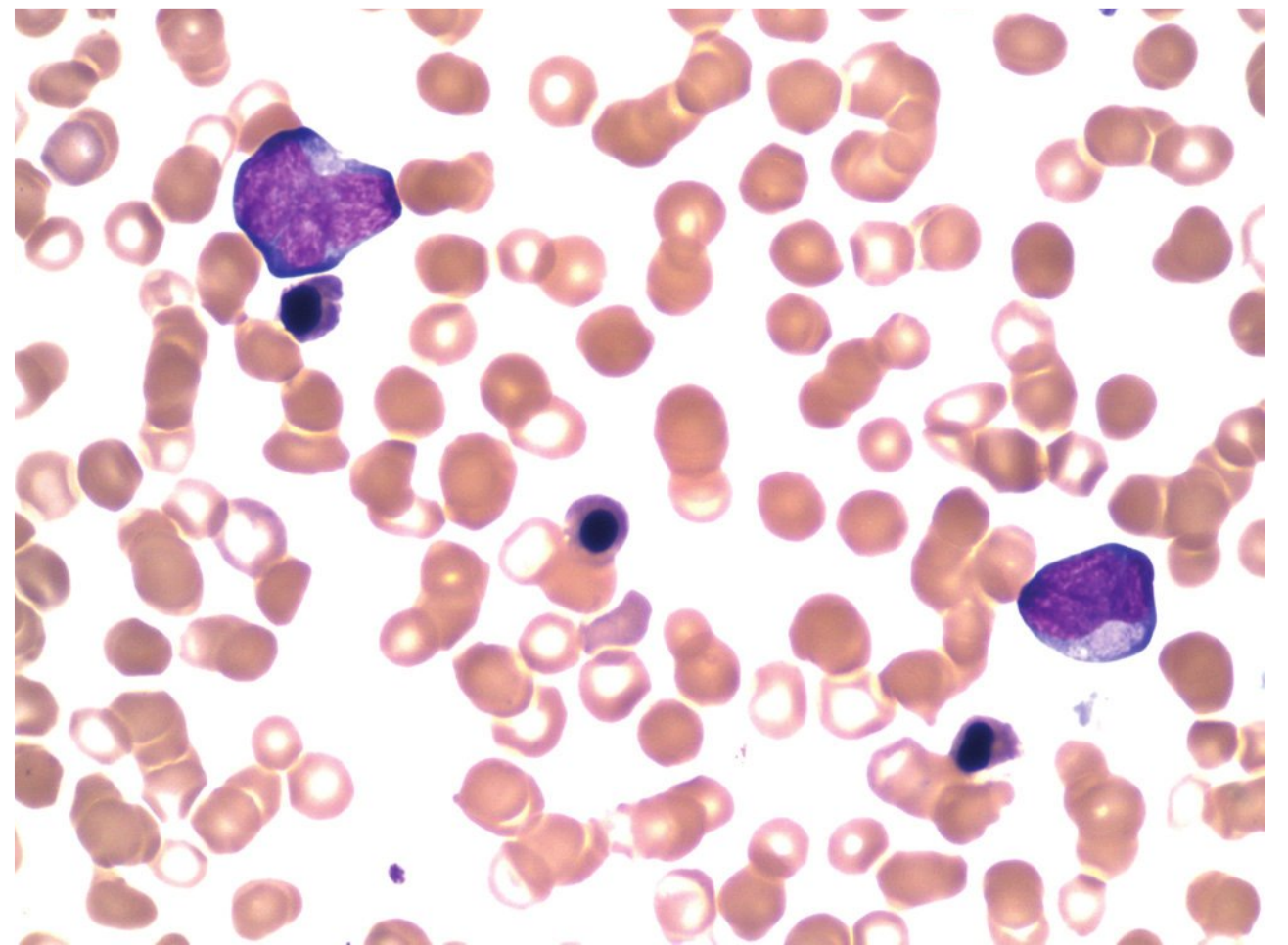


FIGURE 6.2.8 Peripheral blood smear reveals two immature myeloid cells and three nucleated red blood cells (leukoerythroblastosis). Wright–Giemsa, $\times 100$.

The 2008 WHO classification includes the same morphologic criteria as the original scheme, but it further emphasizes the exclusion of PV, chronic myeloid leukemia, myelodysplastic syndromes, or other myeloid neoplasms and the inclusion of molecular genetic markers, such as JAC2 and MPL (Table 6.2.1) (21). It also singles out anemia and lactate dehydrogenase levels in the minor diagnostic criteria that were not mentioned in the original criteria. Although increase of reticulin or collagen fibers is the hallmark of PMF and the WHO classification advocates grading of bone marrow fibrosis (Table 6.2.2) (Fig. 6.2.9) (21), the most distinguishing feature in PMF is the morphology and distribution of megakaryocytes. The megakaryocytes in PMF show a great variation in size with an aberrant nuclear/cytoplasmic ratio and hyperchromatic, bulbous, or irregularly folded nuclei (22) (Fig. 6.2.10). Naked nuclei are also commonly seen. These bizarre megakaryocytes are not seen in the other two entities. These megakaryocytes form dense clusters adjacent to the bone trabeculae or vascular sinuses. As a result of fibrosis, the sinuses are dilated (Fig. 6.2.11) and tortuous blood vessels are characteristically present. Intrasinusoidal hematopoiesis is also a common finding (Fig. 6.2.12). The increase of blasts to 10% to 19% is considered to define an accelerated phase and more than 20%, acute leukemia transformation.

The pathogenesis of myelofibrosis is thought to be the release of cytokines from the megakaryocytes and monocytes. The cytokines frequently mentioned include transforming growth factor beta, platelet-derived growth factor, basic fibroblast growth factor, vascular endothelial growth factor, and tissue inhibitors of matrix metalloproteinases (23).

Immunophenotype

There is no specific immunophenotype for MPN entities. CD34 is used to highlight the blasts in the bone marrow biopsy and to identify the accelerated phase and leukemic transformation. Platelet antibodies, such as CD61,

TABLE 6.2.1

WHO Diagnostic Criteria for PMF

Major criteria

- 1. Presence of megakaryocyte proliferation and atypia, usually accompanied by either reticulin and/or collagen **f**ibrosis or in the absence of significant reticulin **f**ibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis (i.e., pre**f**ibrotic cellular-phase disease).
- 2. Not meeting WHO criteria for PV, BCR-ABL1+ CML, myelodysplastic syndrome, or other myeloid neoplasms.
- 3. Demonstration of JAK2 V617F or other marker (e.g., MPL W515K/L) or in the absence of a clonal marker, no evidence that the bone marrow **f**ibrosis or other changes are secondary to infection, autoimmune disorder or other chronic in**f**lammatory condition, hairy cell leukemia or other lymphoid neoplasm, metastatic malignancy, or toxic (chronic) myelopathies.

Minor criteria

- 1. Leukoerythroblastosis
- 2. Increase in serum lactate dehydrogenase level
- 3. Anemia
- 4. Splenomegaly

TABLE 6.2.2

Semiquantitative Grading of Bone Marrow Fibrosis

Grading	Description*
MF-0	Scattered linear reticulin with no intersections (crossover), corresponding to normal bone marrow
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis
MF-3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis

*Fiber density should be assessed only in hematopoietic areas.

help to demonstrate megakaryocytic dysplasia and the atypical forms. There are a few studies that emphasize the importance of identifying increased CD34-positive cells (>15,000/dL) in the peripheral blood without the increase of the same cells in the bone marrow (11,24,25). This is considered an early sign of PMF.

Molecular Genetics

A breakthrough of our understanding in the molecular pathogenesis of MPN occurred in 2005 when JAK2 mutation was discovered. The mutation in JAK2 substitutes a bulky phenylalanine for a conserved valine at position 617 of the JAK2 protein (V617F). However, because about 5% PV cases were negative for this mutation, further studies were done to investigate whether the JAK-STAT (signal transducers and activators of transcription) signaling pathway was activated by other mutations. As a result, somatic mutations in exon 12 of JAK2 (JAK2-ex12) was identified in JAK2 mutation–negative PV cases (2). However, JAK2-ex12 has not been identified in ET and PMF patients so far. Sequence analysis of JAK-STAT pathway members in JAK2 mutation–negative PMF patients identified two gain-of-function mutations in the thrombopoietin receptor (MPL), including MPL-W515L and MPL-W515K (2). It has a

frequency of about 5% in PMF and between 1% and 9% in ET, but these mutations have not been found in PV cases.

JAK2 V617F has been found in 95% of PV patients and in about 50% to 60% in ET and PMF patients. It has been observed in low frequency in patients with the hypereosinophilic syndrome, chronic myelomonocytic leukemia, chronic neutrophilic leukemia, myelodysplasia, and acute myeloid leukemia and systemic mastocytosis (2,8). However, it can be seen in about 50% of patients with refractory anemia with ringed sideroblasts associated with marked thrombocytosis (26). JAK2 is a cytoplasmic tyrosine kinase, which is important in triggering intracellular signaling by the receptors for erythropoietin, thrombopoietin, interleukin-3,

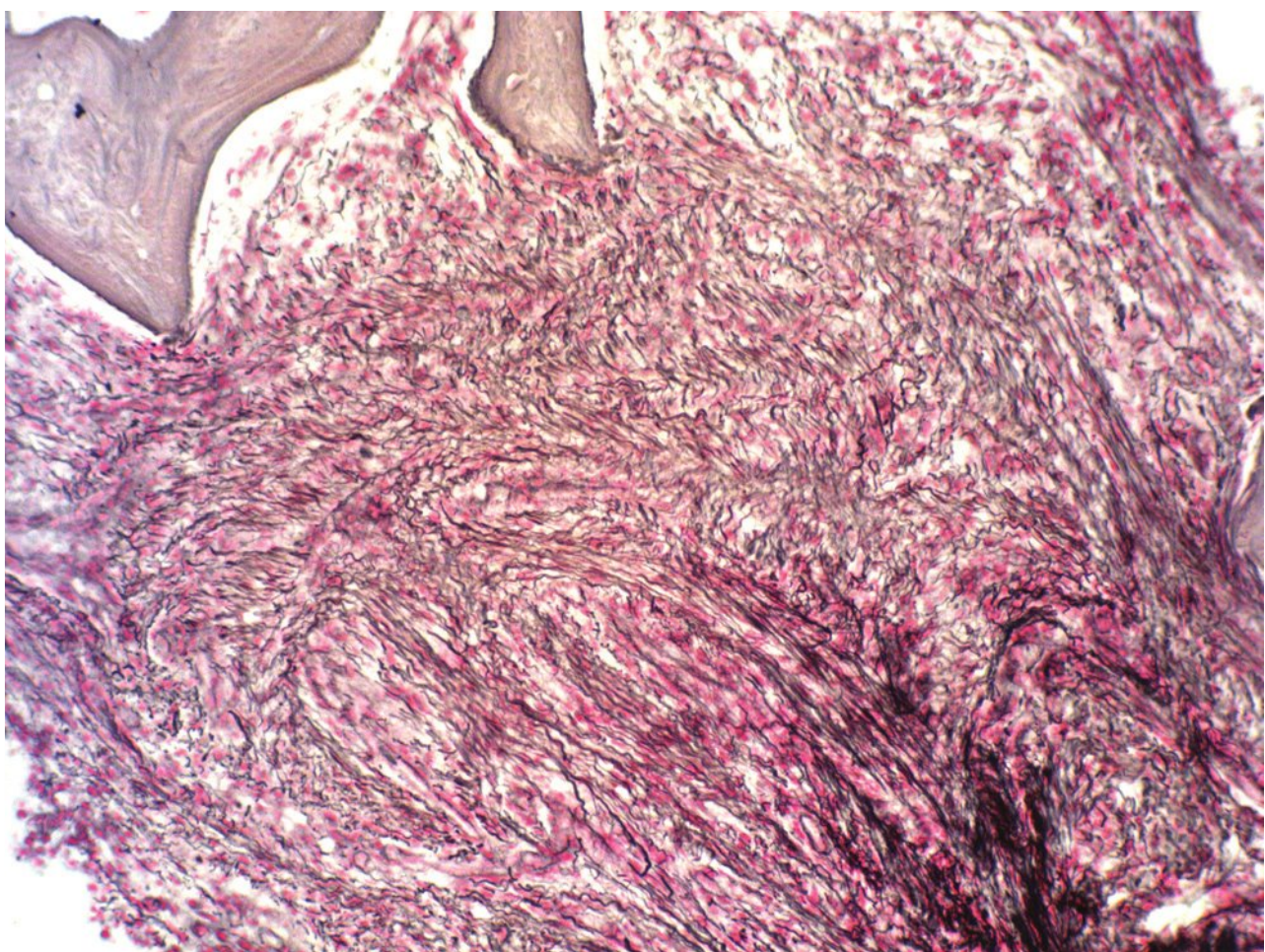


FIGURE 6.2.9 Bone marrow biopsy demonstrates diffuse and dense increase in reticulin **f**ibers with extensive intersections. Reticulin stain, ×40.

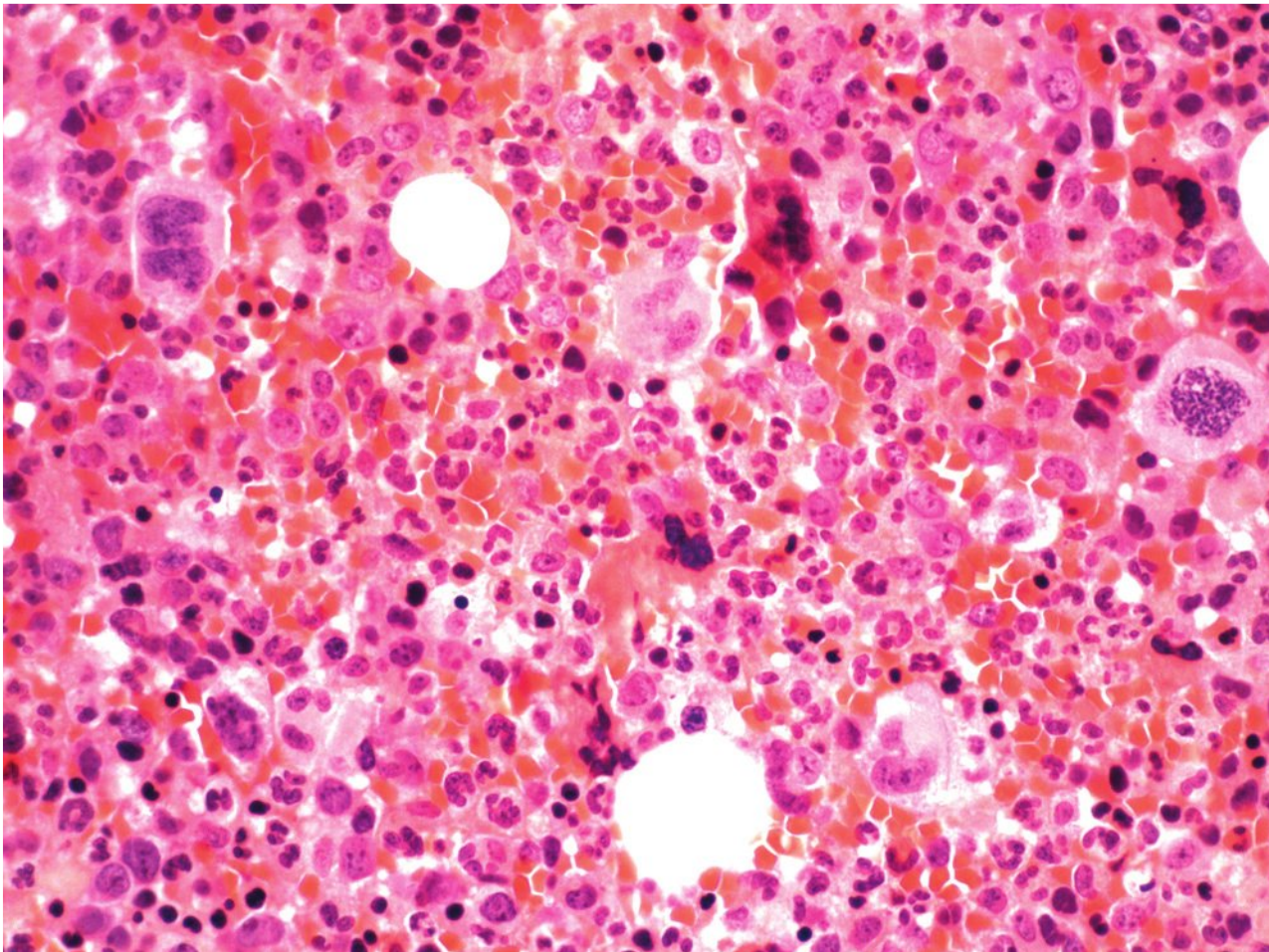


FIGURE 6.2.10 Bone marrow biopsy shows panmyelosis. Note presence of bizarrely shaped and hyperchromatic megakaryocytes, characteristic of PMF. H&E, $\times 40$.

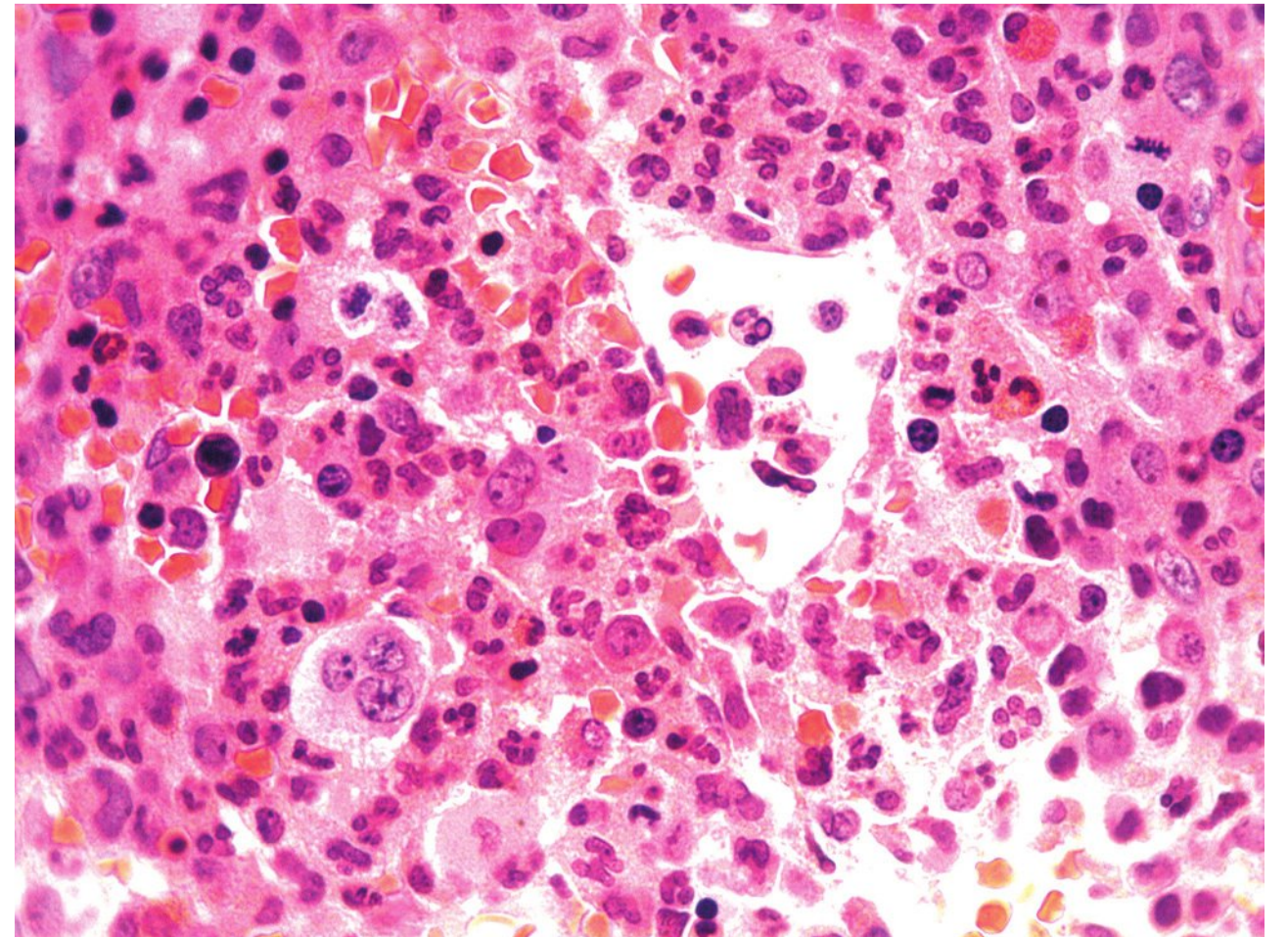


FIGURE 6.2.12 Bone marrow biopsy demonstrates panmyelosis with intrasinusoidal hematopoiesis, representing the pre-fibrotic phase of PMF. H&E, $\times 60$.

granulocyte-colony-stimulating factor (G-CSF), and granulocyte-macrophage CSF (8). JAK2 V617F mutation causes cytokine-independent activation of JAK-STAT, PI3K, and AKT pathways and mitogen-activated protein kinase and extracellular signal-regulated kinase, all of which are involved in erythropoietin-receptor signaling. Normally, when erythropoietin binds to its receptor, it activates the wild-type JAK2 that is bound to the cytoplasmic domain of erythropoietin receptor. The activated JAK2, in turn, phosphorylates the receptor and initiates the intracellular signaling cascade. In cells with JAK2 V617F mutation, the signaling is constitutively increased without the erythropoietin activation (8).

An intriguing question is how a mutation in a single gene can induce three different diseases. There is no clear cut answer, but it may involve the allele burden, zygosity of the mutation, and the proportion of the malignant

and the normal stem cells in the bone marrow (18). For instance, ET usually has a low JAK2 V617F allele burden, heterozygosity for the mutation, and the malignant clone is not predominant in the bone marrow, whereas PV has a high allele burden, homozygosity for the mutation, and predominance of the malignant clone in the bone marrow (18). The development of homozygosity for the JAK2 V617F mutation is a two-step process. The first step is point mutation and the second, uniparental disomy on chromosome 9p (8). The latter is the result of mitotic recombination caused by the exchange of chromosomal DNA between nonsister chromatids during mitosis (27). Other explanations for the one mutation-different disease phenomenon include a different stem cell as the target of the mutation, the unique genetic background of the host, and a pre-JAK2 molecular event (28).

The second important question is whether JAK2 V617F is a primary or secondary event. The first theory is that the mutation simultaneously induces clonal hematopoiesis and the onset of MPN phenotype (27). Then, the constitutive genetic factors determine the eventual development of a particular MPN entity. The second theory implies a multi-hit pathogenesis of MPN (27). Mutations or other genetic events acquired before JAK2 V617F occur and establish clonal hematopoiesis. These pre-JAK2 mutations determine which MPN entity will develop in the patient. The second theory is supported by the presence of clonal cells other than JAK2 mutation-positive cells in MPN patients, presence of endogenous erythroid colonies with wild-type JAK2 in MPN patients with JAK2 mutation, presence of del(20q) in JAK2 V617F-positive MPN cases, and family members of MPN patients have inherited predisposition to acquiring somatic mutations in JAK2 (8,28). However, the coexistent mutations may represent two independent clones rather than a sequential clonal evolution. For instance, JAK2, MPL, or JAK-ex12 may be coexistent in the same patient (28).

Among the MPN entities, the most frequent karyotypic aberrations are del(20q), del(13q), +8, +9, and

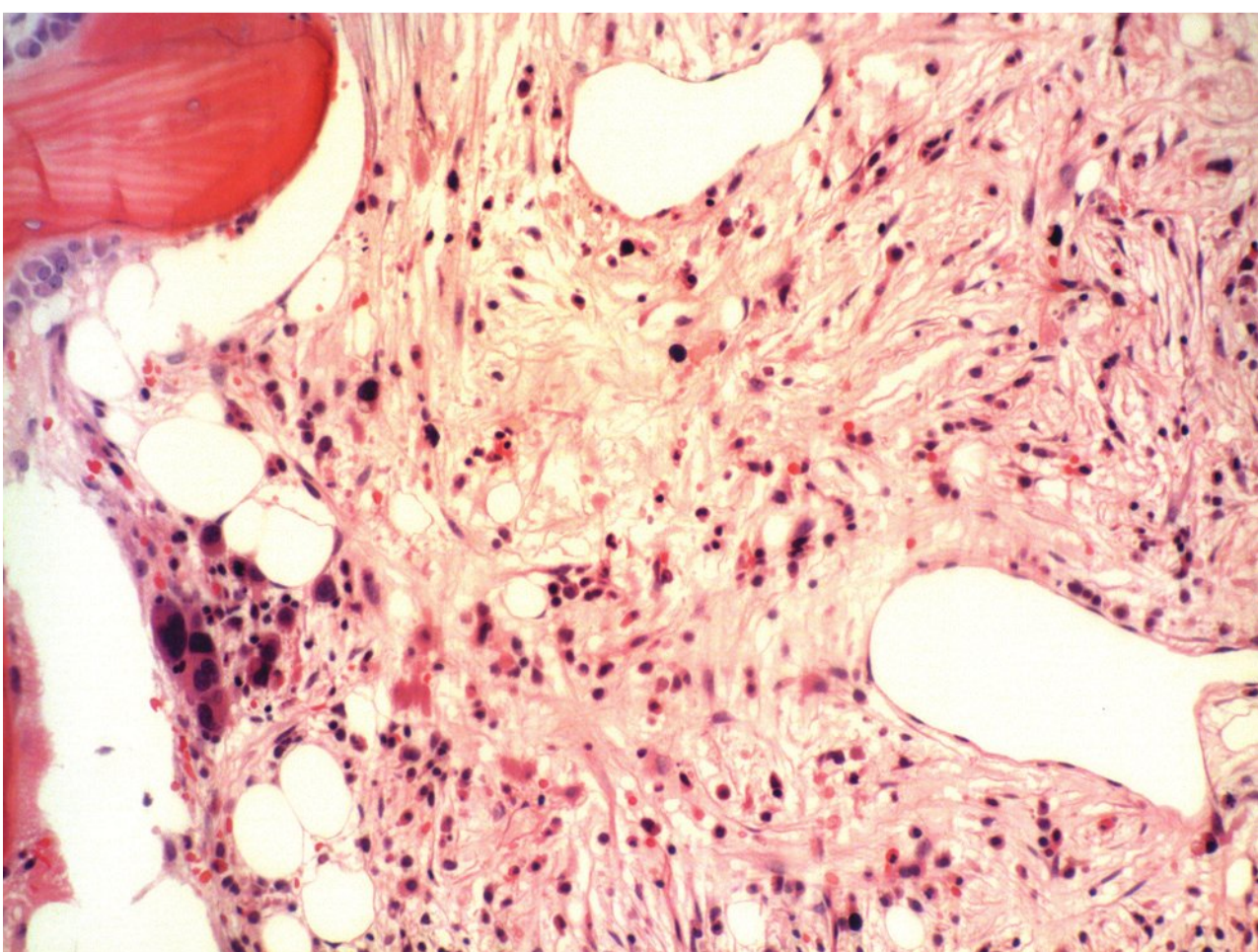


FIGURE 6.2.11 Bone marrow biopsy reveals myelofibrosis with dilatation of sinuses and paratrabecular localization of a cluster of megakaryocytes. H&E, $\times 10$.

abnormalities of chromosomes 1 and 7 (29). In PMF, del(20q), del(13q), and chromosome 1 abnormalities are most common (29). In PV, trisomy 8 and trisomy 9 are demonstrated in a high percentage of cases (29). One study showed chromosome 1 anomalies in 70% of post-PV myelofibrosis cases (30). Deletion of 5q is also frequently associated with myelofibrosis, but cases with this abnormality should be distinguished from myelodysplastic syndromes, which also carry this aberration (19,29). In general, cytogenetic anomalies seen in post-PV/ET myelofibrosis are similar to those seen in PV and PMF, but post-PV cases usually have a higher frequency of abnormal karyotypes than PV cases, as the former represents disease progression (7).

The current case had a history of PV and then developed splenomegaly and hepatic cirrhosis. His bone marrow biopsy was consistent with post-PV myelofibrosis. He had characteristic complications of MPN with portal vein thrombosis and hemorrhage episodes and finally succumbed to gastric bleeding.

Clinical Manifestations

The medium age of PV patients is 60 years with slight male predominance. The clinical presentation is usually associated with increased red cell mass, so that hypertension and vascular symptoms are most common (31,32). Venous or arterial thrombosis is encountered in about 20% of patients with resultant deep vein thrombosis, myocardial ischemia, or stroke. Other clinical manifestations include mesenteric, portal, or splenic vein thrombosis and Budd–Chiari syndrome. The latter is especially common in pediatric patients (33). Constitutional symptoms include headache, dizziness, visual disturbances, paresthesias, pruritus, erythromelalgia, and gout (7). Physical examination may show plethora and splenomegaly in 70% and hepatomegaly in 40% of PV patients.

The WHO diagnostic criteria for PV are listed in Table 6.2.3 (7). The major criteria include (a) a hemoglobin level >18.5 g/dL in man and >16.5 g/dL in women and (b) presence of JAK2 V617F or JAK2-ex12 mutation. The WHO criteria also accept the measurement of red cell mass in place of hemoglobin levels, but the former technique is too complicated to perform in a routine clinical laboratory. The minor WHO criteria include bone marrow biopsy to demonstrate features as mentioned before, a subnormal level of serum EPO, and endogenous erythroid colony formation in vitro. A definitive diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria.

Most ET patients are in the age range of 50 to 60 years with no gender predilection. Many patients are diagnosed incidentally during a routine peripheral blood examination, as most of them are asymptomatic even the platelet count is at a high level. The major clinical presentation is vascular occlusion or hemorrhage (19). Bleeding usually involves the mucosal surfaces, such as the gastrointestinal or respiratory tract. When microvascular occlusion occurs, the patient may have transient ischemic attacks, digital ischemia with paresthesias, and gangrene. Thrombosis is also seen in major arteries and veins, such as splenic or

TABLE 6.2.3

WHO Diagnostic Criteria for PV

Major criteria

1. Hemoglobin >18.5 g/dL in men, 16.5 g/dL in women or other evidence of increased red cell volume
2. Presence of JAK2 V617F or other functionally similar mutation such as JAK2 exon 12 mutation

Minor criteria

1. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) with prominent erythroid, granulocytic, and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro

hepatic vein thrombosis as seen in Budd–Chiari syndrome. With the new diagnostic criteria, splenomegaly is found in only a small percentage of patients.

The WHO diagnostic criteria for ET are listed in Table 6.2.4 (19), which include persistent thrombocytosis >450,000/mL, specific features in bone marrow biopsy, exclusion of PV, PMF, chronic myeloid leukemia, myelodysplastic syndrome or other myeloid neoplasms, and the demonstration of JAK2 V617F or other clonal markers, such as MPL W515K/L (19). All four criteria have to be met for a definitive diagnosis. Exclusion of PV requires the measurement of hemoglobin and hematocrit levels. If hemoglobin is not high, iron treatment should be initiated to make sure it is not falsely low due to iron deficiency. PMF and myelodysplastic syndrome should be ruled out by bone marrow biopsy. BCR-ABL1 testing is required for the exclusion of chronic myeloid leukemia.

Patients with PMF are usually in the sixth to seventh decade of life with nearly equal frequency in both sexes. As mentioned before, PMF is sometimes difficult to distinguish from post-PV/ET as they may show similar peripheral blood features (21,34). PMF patients may be asymptomatic in the early stage and are frequently discovered by physical detection of splenomegaly or abnormal blood counts. Thrombocytosis and anemia are frequently seen, but the most characteristic feature is leukoerythroblastosis; that is the presence of nucleated red blood cells and immature granulocytes in the peripheral blood smear. The above features and increase in serum lactate dehydrogenase level are all included as the minor criteria in the WHO classification (Table 6.2.1) (21). The major diagnostic criteria include the characteristic bone marrow morphology, exclusion of chronic myeloid leukemia, myelodysplastic syndrome or other myeloid neoplasms, and demonstration of JAK2 V617F or other clonal marker, such as MPL W515K/L. For a definitive diagnosis of PMF, all three major criteria and two

TABLE 6.2.4

WHO Diagnostic Criteria for ET

1. Sustained platelet count $\geq 450 \times 10^9/L$
2. Bone marrow biopsy specimen showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left shift of neutrophil granulopoiesis or erythropoiesis
3. Not meeting WHO criteria for polycythemia vera, primary myelofibrosis, BCR-ABL1-positive chronic myelogenous leukemia, or myelodysplastic syndrome or other myeloid neoplasm
4. Demonstration of JAK2 V617F or other clonal marker, or in the absence of JAK2 V617F, no evidence for reactive thrombocytosis

minor criteria should be met. These patients may have constitutional symptoms, including fatigue, dyspnea, weight loss, night sweats, low-grade fever, and bleeding episodes. Because of hyperuricemia, gouty arthritis or renal stones are found in some patients. Splenomegaly is more frequently demonstrated and more prominent than in other MPN entities. About half of the patients may have hepatomegaly.

ET and PV are usually associated with an indolent clinical course, low-risk patients may survive for 20 or more years without cytoreductive therapy (35). The high-risk patients with ET or PV can be well managed by treatment with hydroxyurea, low-dose aspirin, and phlebotomy in case of PV (35). On the other hand, PMF patients have a medium survival of <5 years and require more aggressive therapy than the other two entities (35). The prospect of JAK2-targeted therapy in MPNs is an exciting new development, but further studies are required. Therapeutic response criteria for MPNs have recently been established in a European leukemia consensus conference, which provides a standard for international comparison of therapeutic effects of various approaches (36).

REFERENCES

1. Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood*. 1951;6:372–375.
2. Kilpivaara O, Levine RL. JAK2 and MPL mutations in myeloproliferative neoplasms: discovery and science. *Leukemia*. 2008;22:1813–1817.
3. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2008;22:14–22.
4. Reilly JT. Pathogenetic insight and prognostic information from standard and molecular cytogenetic studies in the BCR-ABL-negative myeloproliferative neoplasms (MPNs). *Leukemia*. 2008;22:1818–1827.
5. Tefferi A. JAK and MPL mutations in myeloid malignancies. *Leuk Lymphoma*. 2008;49:388–397.
6. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001.
7. Thiele J, Kvasnicka HM, Orazi A, et al. Polycythemia vera. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:40–43.
8. Campbell PJ, Green AR. The myeloproliferative disorders. *N Engl J Med*. 2006;355:2452–2466.
9. Wasserman LR. The treatment of polycythemia. A panel discussion. *Blood*. 1986;32:483–487.
10. Michels JJ, Juvonen E. Proposal for revised diagnostic criteria of essential thrombocythemia and polycythemia vera by the Thrombocythemia Vera Study Group. *Semin Thromb Hemost*. 1997;23:339–347.
11. Spivak JL, Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocytosis and primary myelofibrosis: an alternative proposal. *Blood*. 2008;112:231–239.
12. Kvasnicka HM, Thiele J. Prodromal myeloproliferative neoplasms: the 2008 WHO classification. *Am J Hematol*. 2010;85:62–69.
13. Gianelli U, Iurlo A, Vener C, et al. The significance of bone marrow biopsy and JAK2-V617F mutation in the differential diagnosis between the “early” prepolycythemic phase of polycythemia vera and essential thrombocythemia. *Am J Clin Pathol*. 2008;130:338–342.
14. Thiele J, Kvasnicka HM, Diehl V. Initial (latent) polycythemia vera with thrombocytosis mimicking essential thrombocythemia. *Acta Haematol*. 2005;113:213–219.
15. Thiele J, Kvasnicka HM. Diagnostic impact of bone marrow histopathology in polycythemia vera (PV). *Histol Histopathol*. 2005;20:317–328.
16. Geogii A, Buesche G, Kreft A. The histopathology of chronic myeloproliferative diseases. *Baillieres Clin Haematol*. 1998;11:721–749.
17. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. *Leukemia*. 2009;23:834–844.
18. Zhan H, Spivak JL. The diagnosis and management of polycythemia vera, essential thrombocythemia, and primary myelofibrosis in the JAK2 V617F era. *Clin Adv Hematol Oncol*. 2009;7:334–342.
19. Thiele J, Kvasnicka HM, Orazi A, et al. Essential thrombocythemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:48–50.
20. Barosi G, Ambrosetti A, Finelli C, et al. The Italian consensus conference on diagnostic criteria for myelofibrosis with myeloid metaplasia. *Br J Haematol*. 1999;104:730–737.
21. Thiele J, Kvasnicka HM, Tefferi A, et al. Primary myelofibrosis. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:44–47.
22. Tefferi A, Thiele J, Orazi A, et al. Proposals and rationale for revision of the World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: recommendations from an ad hoc international expert panel. *Blood*. 2007;110:1092–1097.
23. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. *J Clin Oncol*. 2005;23:8520–8530.
24. Barosi G, Viarengo G, Pecci A, et al. Diagnostic and clinical relevance of the number of circulating CD34+ cells in myelofibrosis and myeloid metaplasia. *Blood*. 2001;98:3249–3255.

25. Passamonti F, Vanelli L, Malabarba L, et al. Clinical utility of the absolute number of circulating CD34-positive cells in patients with chronic myeloproliferative disorders. *Haematologica*. 2003;88:1123–1129.
26. Szpurka H, Tiu R, Murugesan G, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JACK2 V617F mutation. *Blood*. 2006;108:2173–2181.
27. Kralovics R. Genetic complexity of myeloproliferative neoplasms. *Leukemia*. 2008;22:1841–1848.
28. Goldman JM, Green AR, Holyoake T, et al. Chronic myeloproliferative diseases with and without the Ph chromosome: some unresolved issues. *Leukemia*. 2009;23:1708–1715.
29. Hussein K, Van Dyke DL, Tefferi A. Conventional cytogenetics in myelofibrosis: literature review and discussion. *Europ J Haematol*. 2009;82:329–338.
30. Andrieux J, Demory JL, Caulier MT, et al. Karyotypic abnormalities in myelofibrosis following polycythemia vera. *Cancer Genet Cytogenet*. 2003;140:118–123.
31. Orlandi E, Castelli G, Brusamolino E, et al. Hemorrhagic and thrombotic complications in polycythemia vera. A clinical study. *Haematologica*. 1989;74:45–49.
32. Anonymous. Polycythemia vera: the natural history of 1213 patients followed for 20 years. Gruppo Italiano Studio Policitemia. *Ann Intern Med*. 1995;123:656–664.
33. Carlo H, McMullin MF, Pahl HL. Clinical and hematological presentation of children and adolescents with polycythemia vera. *Ann Hematol*. 2009;88:713–719.
34. Abdel-Wahab OI, Levine RL. Primary myelofibrosis: Update on definition, pathogenesis, and treatment. *Annu Rev Med*. 2009;60:233–245.
35. Tefferi A. Essential thrombocythemia, polycythemia vera, and myelofibrosis: current management and the prospect of targeted therapy. *Am J Hematol*. 2008;83:491–497.
36. Barosi G, Birgegard G, Finazzi G, et al. Response criteria for essential thrombocythemia and polycythemia vera: result of a European LeukemiaNet Consensus conference. *Blood*. 2009;113:4829–4833.

CASE 3

Myelodysplastic Syndromes

CASE HISTORY

An 81-year-old man was admitted because of abdominal pain and diarrhea for 4 days. The patient claimed that he had had anemia for about 20 years, but he had been asymptomatic until several months prior to admission when he developed shortness of breath, fatigue, and palpitations. He was then found to have pancytopenia, and a bone marrow examination revealed hypocellular bone marrow that was consistent with aplastic anemia. Initial workups, including vitamin B₁₂, folate, and antinuclear antibody screening, were all within normal limits. The patient was a sheet-metal worker with frequent exposure to paint sprays.

Physical examination showed lower abdominal pain localized in the suprapubic area with hyperactive bowel sounds. There was no hepatosplenomegaly, and no peripheral lymph node was palpable. The skin demonstrated no petechiae, ecchymoses, or purpura.

Hematologic workup showed a total leukocyte count of 1,200/mL with 29.9% neutrophils, 60.4% lymphocytes, 5.7% monocytes, 3.2% eosinophils, and 0.8% basophils. The hematocrit was 28%, hemoglobin 9.8 g/dL, mean cell volume 103.1 fL, and platelets 40,000/mL. The bone marrow aspirate revealed erythroid and megakaryocytic dysplasia with the presence of 12% myeloblasts.

The patient was treated with intravenous fluids with prompt improvement of abdominal symptoms. He responded well with subsequent administration of erythropoietin and granulocyte-colony-stimulating factor (G-CSF; Neupogen). His peripheral blood cell count before

discharge showed a total leukocyte count of 7,600/ μ L with an absolute neutrophil count of 6,400/mL. His hematocrit was 34.2% and platelets 65,000/mL.

FLOW CYTOMETRY FINDINGS

In the bone marrow aspirate, the following results were obtained: T-cell marker: CD7, 0%. B-cell marker: CD19, 0%. Myeloid markers: CD13-CD33, 84%; CD14, 4%; myeloperoxidase, 30%. Major histocompatibility complex-II antigen: HLA-DR, 76%. Stem cell markers: CD34, 50%; CD117, 55% (Fig. 6.3.1).

CYTOGENETIC FINDING

Cytogenetic analysis of unstimulated cultures revealed an apparently normal GTG banding pattern: 46, XY.

DISCUSSION

Myelodysplastic syndromes (MDS) are a heterogeneous group of disorders with ineffective hematopoiesis and myeloid dysplastic changes in the bone marrow and peripheral blood. As a result, the bone marrow is usually hypercellular and the peripheral blood is cytopenic in one or more cell lineages.

MDS are usually seen in elderly persons, who may have normal hematopoiesis under normal condition but

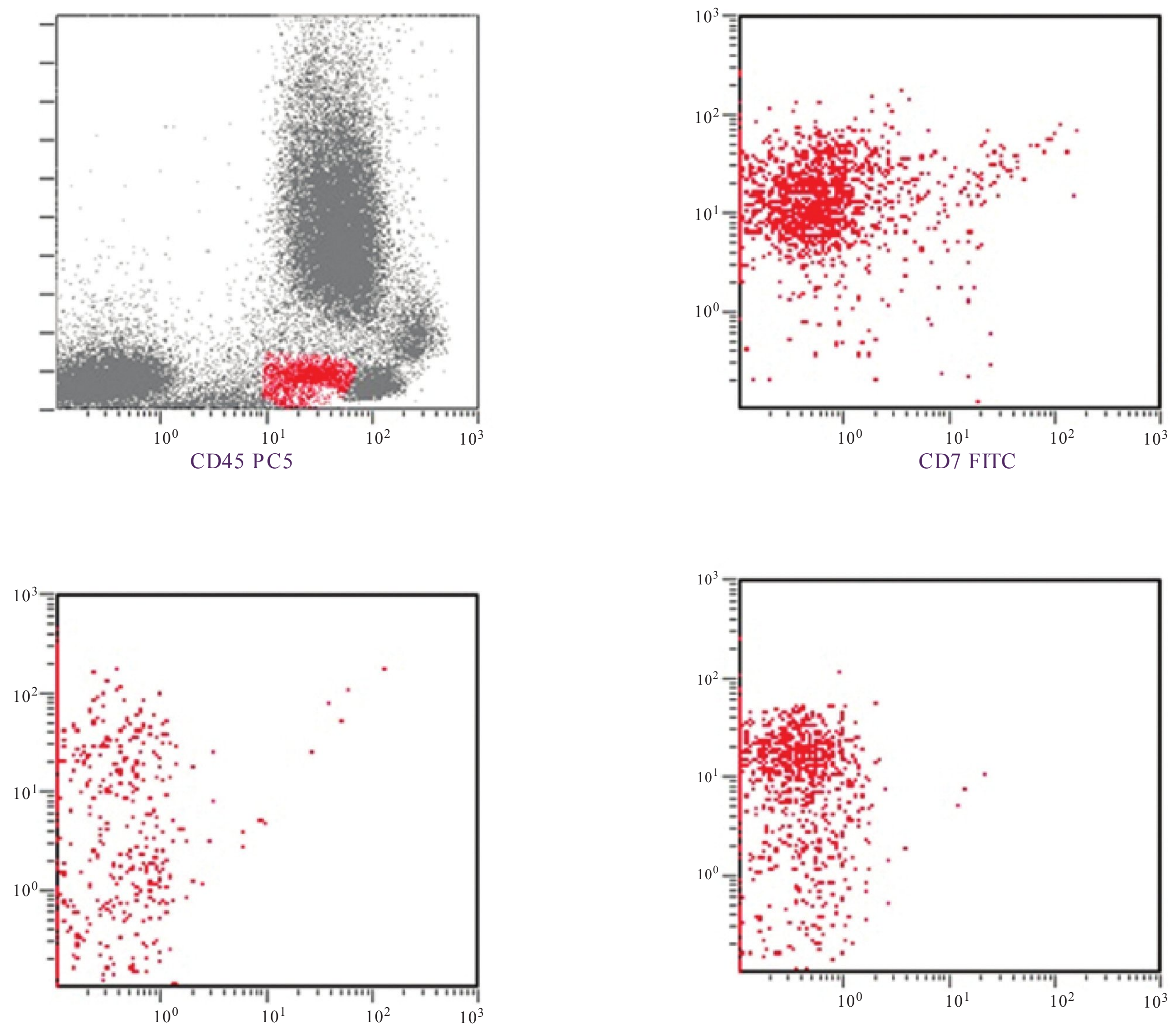


FIGURE 6.3.1 Flow cytometric histograms show the gating of an immature myeloid population with positive reactions to CD13, CD33, CD34, and CD117. This cluster represents the myeloblasts in a case of RAEBs. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

may have latent age-associated defects that may lead to the development of MDS when under stress (1). The pathogenesis of MDS may be multifactorial, but apoptosis of myeloid cells before their maturation and release from the bone marrow may play an important role (1,2). Other factors include deficiency in humoral promoters, damage of the microenvironment, loss of the ability of progenitors to respond to stimuli, and replacement of the normal marrow with abnormal clones of hematopoietic cells (1).

The French-American-British (FAB) classification of MDS was first established in 1982 and divided MDS into five categories: (a) refractory anemia (RA), (b) RA with ringed sideroblasts (RARS), (c) RA with excess blasts (RAEB), (d) RAEB in transformation (RAEB-T), and (e) chronic myelomonocytic leukemia (CMML) (3).

The World Health Organization (WHO) classification modified the old classification into six categories (4–7). First, the blast count for acute myelogenous leukemia (AML) in the FAB classification was 30%, but recent studies have found that patients with 20% to 30% blasts have the same prognosis as those patients with >30% blasts. In addition, 50% to 60% of patients with RAEB-T evolve to AML within 6 months after initial diagnosis (7). Therefore, the subtype of RAEB-T is now classified as AML. Second, CMML has features of both MDS and myeloproliferative disorders, so that it is now classified under myelodysplastic/myeloproliferative neoplasms. Third, on the basis of the blast count, RAEB is further divided into RAEB-1 and RAEB-2 categories. Finally, three new categories were added in the 2001 WHO classification: refractory cytopenia with multilineage

TABLE 6.3.1

Differences in Various Subtypes of MDS

Type	Blasts in marrow (%)	Blasts in blood (%)	Ring sideroblasts (%)	Dysmyelopoiesis
RCUD	>5	<1	<15	Erythroid, neutrophil, or megakaryocyte
RARS	<5	No blast	>15	Erythroid
RAEB1	5–9	2–4	Variable	Unilineage or multilineage
RAEB2	10–19	5–19	Variable	Unilineage or multilineage
RCMD	<5	<1	Variable	Multilineages

MDS, myelodysplastic syndrome; RCUD, refractory cytopenia with unilineage dysplasia; RARS, refractory anemia with ring sideroblasts; RAEB, refractory anemia with excess blasts; RCMD, refractory cytopenia with multilineage dysplasia.

dysplasia (RCMD); 5q- syndrome; and MDS, unclassifiable (MDS-U). In the 2008 WHO scheme, two more new entities, refractory cytopenia with unilineage dysplasia (RCUD) (including the old entity of RA) and refractory cytopenia of childhood (RCC), have been added (8).

Morphology

The criteria for classification are based on both quantitative and qualitative changes (Table 6.3.1) (4–14). Quantitatively, the major parameters are the percentages of blasts and monocytes in the bone marrow and the peripheral blood and the percentage of ring sideroblasts among the erythrocyte precursors. Qualitatively, it is the dysplastic changes seen in different cell lineages. Dysplasia is mainly manifested as the changes of the configuration and lobulation of the nuclei, the size of the nuclei and of the entire cell, and cytoplasmic granularity.

In the erythroid series, the most common findings are megaloblastoid changes (Fig. 6.3.2) and the presence of ring sideroblasts (Fig. 6.3.3), which is due to the deposition of iron in the mitochondria of normoblasts. A ring sideroblast is defined by ≥ 5 iron granules encircling one third or more of the nuclear circumference in an iron-stained smear (10). The nuclear configuration can be in a bizarre shape (e.g., budding, internuclear bridging), multilobated, fragmented, or karyorrhetic (Fig. 6.3.4). The cytoplasm may contain inclusions, such as Howell–Jolly bodies and Pappenheimer bodies or vacuoles. The normoblasts may become periodic acid-Schiff (PAS) positive in contrast to the negative staining in normal nucleated red blood cells. Anisocytosis, poikilocytosis, and nucleated red blood cells may be seen on the peripheral blood smears.

In the granulocytic series, the most common findings are hypolobulation and hypogranularity. When a nonlobated or bilobed nucleus is present, those cells are referred to as pseudo-Pelger–Huet cells (Fig. 6.3.5). Hypersegmentation (Fig. 6.3.6), hypergranularity, giant nuclei, or huge cell size are also features of myeloid dysplasia, if vitamin B₁₂ and folate deficiency are excluded. Bizarre nuclear configuration, ring granulocytic nucleus (Fig. 6.3.7), nuclear fragmentation, and separated nuclear lobes may also occur

in some cases. The presence of pseudo-Chediak–Higashi granules has been reported, but this finding is extremely rare. Auer rods are present in rare cases.

In the megakaryocytic series, the most common findings are micromegakaryocytes, hypolobulation, monolobulation, and the presence of naked nuclei (Fig. 6.3.8). The nuclei may be arranged in a bizarre pattern or in widely separated lobes. Hypogranular and multinucleated megakaryocytes can also be demonstrated in some cases.

All of these dysplastic features should be demonstrated in a high-quality and freshly prepared blood or bone marrow smear. If a smear is made >2 hours after specimen collection, the cell morphology can be distorted and it is invalid for estimation of myelodysplasia. Myelodysplastic changes can be seen in many different conditions, such as vitamin B₁₂ or folate deficiency, heavy metal exposure, paroxysmal nocturnal hemoglobinuria, treatment with G-CSF, and congenital hematologic disorders (4). Therefore, a diagnosis of MDS should not be made until other possible causes are excluded. A few dysplastic cells can sometimes be seen in normal persons; thus, at least 10% dysplastic cells should be identified in a particular cell lineage before it is called MDS. In some cases of unilineage cytopenia, a diagnosis is difficult to make; those cases should be observed for 6 months before calling it MDS (5).

Histologic examination of core biopsy is not as helpful as aspirate in providing positive identification of MDS. The most distinguishing feature of MDS in tissue sections is the so-called abnormal localization of immature precursors (ALIPs), which is usually presented in high-grade MDS and is associated with a more rapid evolution to acute myeloid leukemia (4,15,16). In normal hematopoiesis, the immature myeloid cells first appear along the paratrabecular zone and gradually move to the intertrabecular area as they become mature. The definition of ALIP is the presence of at least three aggregates of three to more than five myeloblasts and promyelocytes in the intertrabecular area (Fig. 6.3.9). On the contrary, erythroid or megakaryocytic precursors are normally present centrally in the bone marrow. Therefore, the

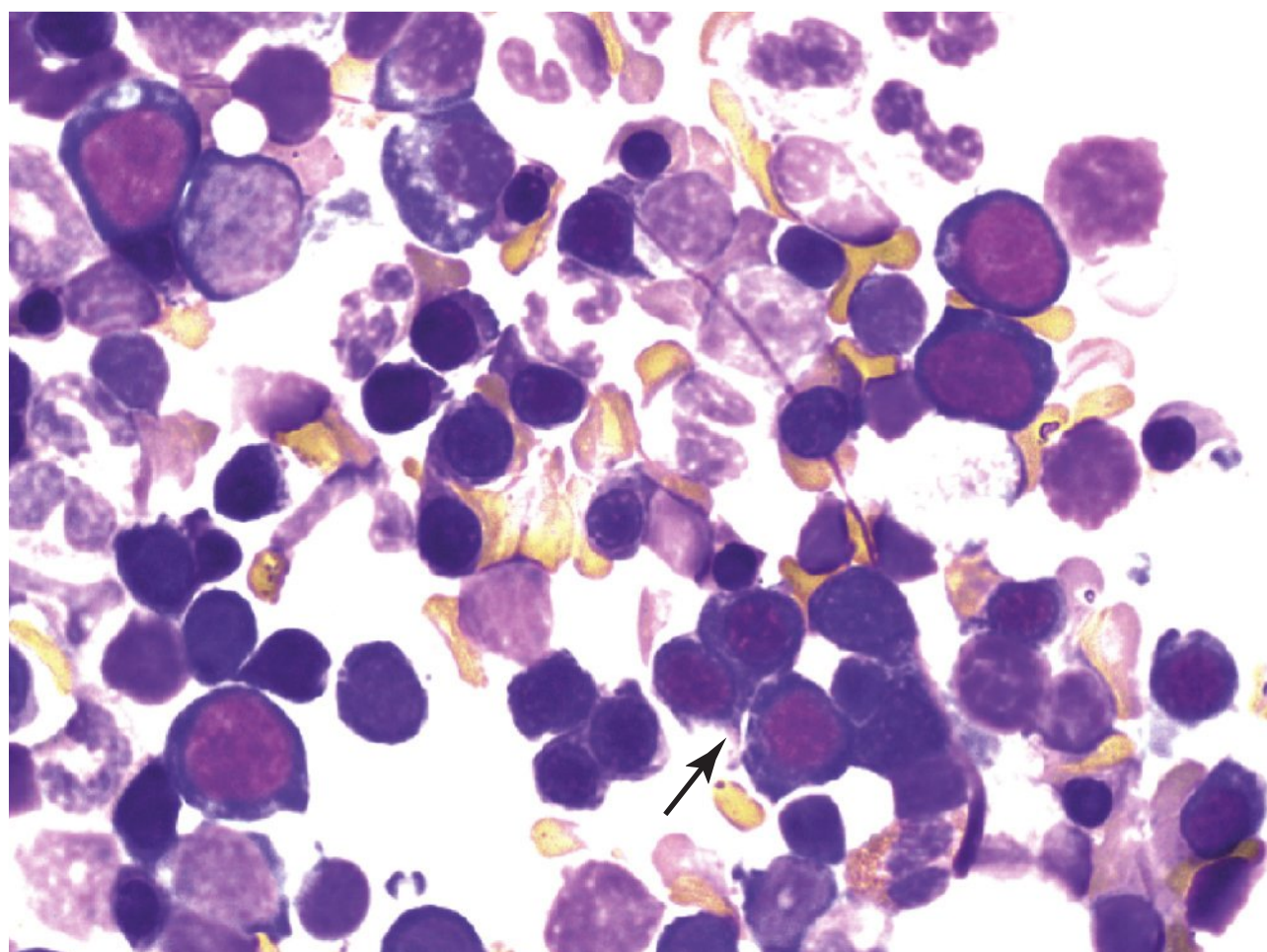


FIGURE 6.3.2 Bone marrow aspirate from a case of RA shows erythroid hyperplasia with the presence of a cluster of megaloblastoid normoblasts (arrow). Wright-Giemsa stain, 100 magnification.

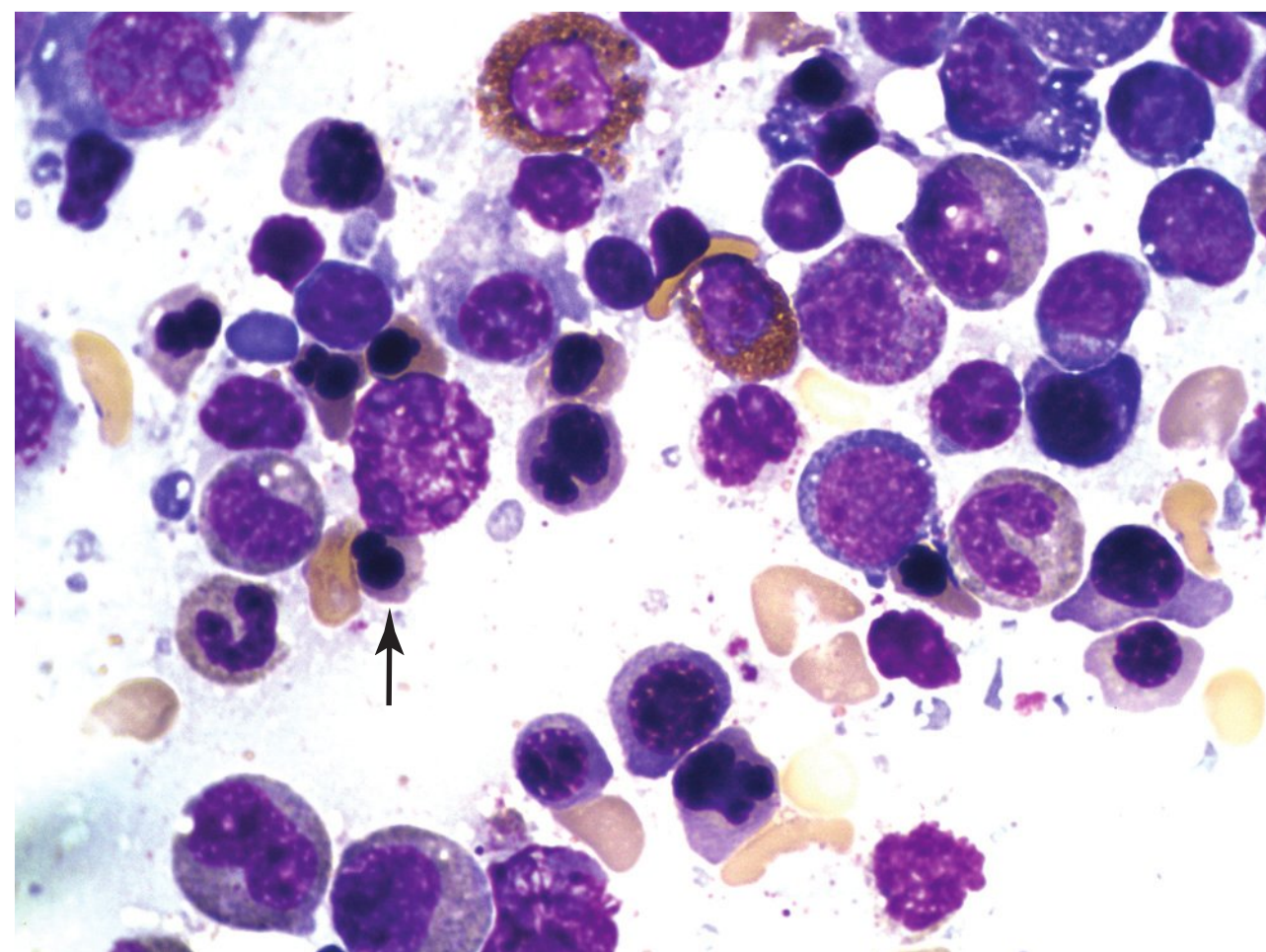


FIGURE 6.3.4 Bone marrow aspirate from a case of RAEBS shows many dysplastic normoblasts with nuclear budding or bizarre nuclear shapes (arrow). Wright-Giemsa stain, 100× magnification.

detection of clusters of pronormoblasts and immature megakaryocytes in the intertrabecular areas is called pseudo-ALIP (Fig. 6.3.10). In contrast, it is abnormal to find erythroid precursors and megakaryocytes concentrate in the paratrabecular region, which can be seen in MDS. Although ALIP is characteristic of MDS, it can also be seen in chronic myeloproliferative disorders, post-transplantation bone marrow, or in patients receiving granulocyte growth factors (13).

Cellularity is best evaluated by bone marrow biopsy. Most MDS cases have hypercellularity, and the minority has normocellularity. When the cellularity is <30% in patients <60 years or <20% in patients >60 years, it is classified as hypocellular MDS, which is highly responsive to immunosuppressive therapy (6).

Microscopic examination of the spleen in 13 MDS cases showed four histologic patterns, erythrophagocytosis, extramedullary hematopoiesis, red pulp plasmacytosis, and red pulp monocytosis (17).

Refractory Cytopenia with Unilineage Dysplasia

This entity is defined by the presence of RCUD, including refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT).

Refractory Anemia RA mainly affects the erythroid series. The anemia is usually normochromic and macrocytic but may be normocytic. The granulocytes and platelets are generally normal, but neutropenia and thrombocytopenia may occur in some patients. Blasts are seen in <1%

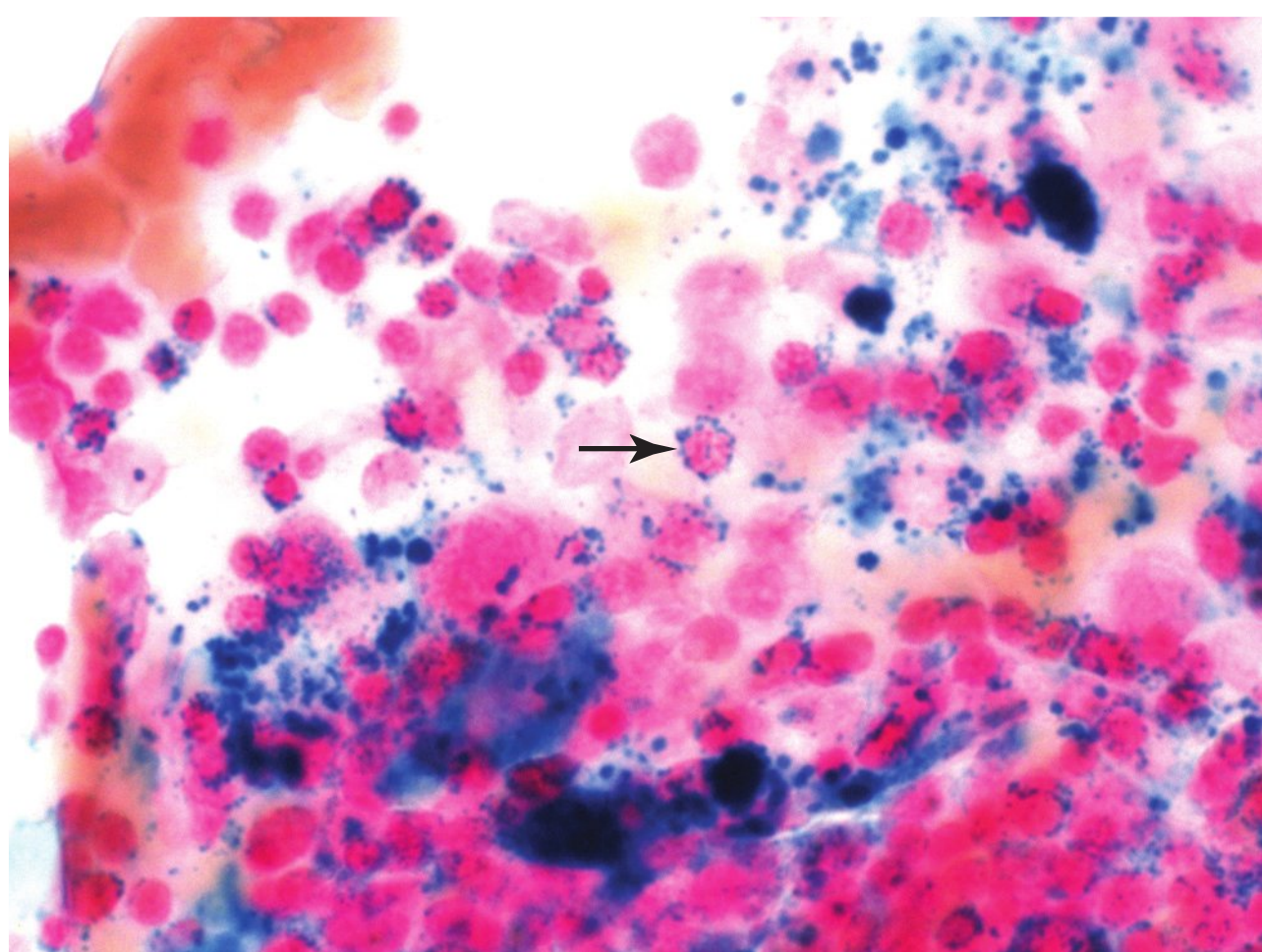


FIGURE 6.3.3 Bone marrow aspirate from a case of RARSs shows many RSs (arrow) in the Prussian blue-stained smear. 100× magnification.

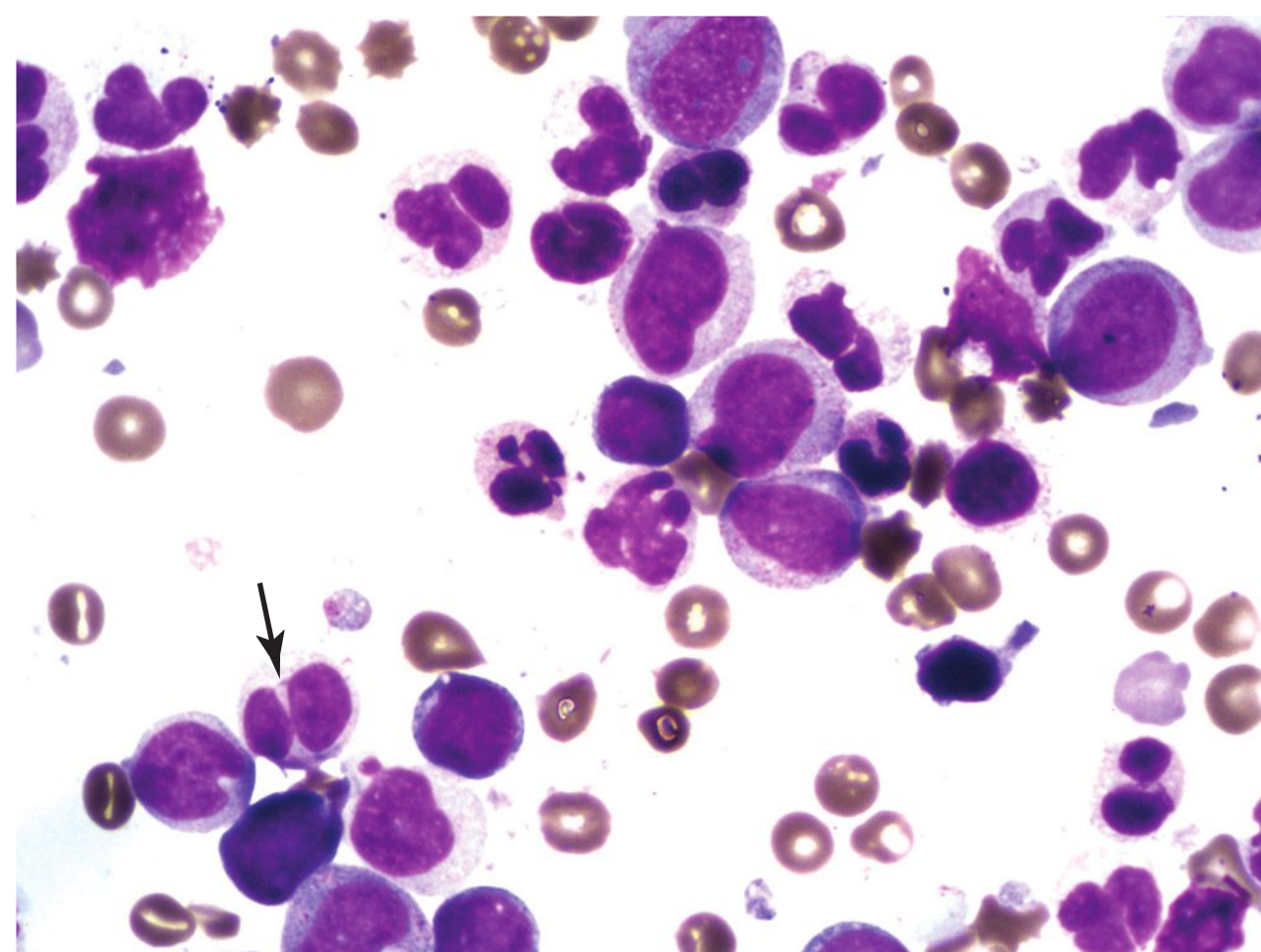


FIGURE 6.3.5 Bone marrow aspirate from a case of RCMD shows several hypolobated pseudo-Pelger-Huet cells (arrow). Wright-Giemsa stain, 100× magnification.

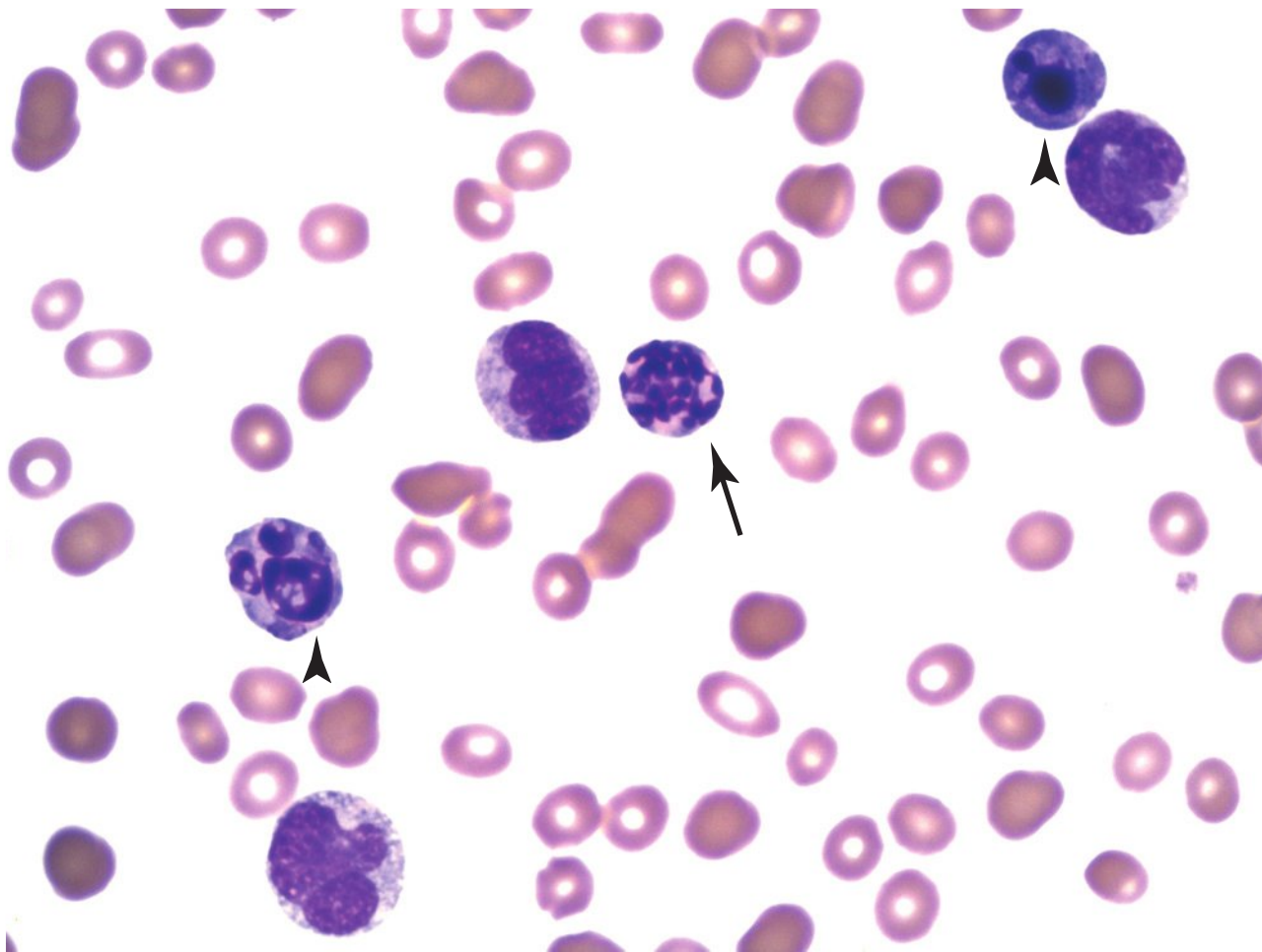


FIGURE 6.3.6 Bone marrow biopsy from a case of MDS, unclassifiable, shows a hypersegmented neutrophil (arrow) and two apoptotic cells (arrowheads). Wright-Giemsa stain, 100× magnification.

in the peripheral blood and $<5\%$ in bone marrow. The bone marrow is usually hypercellular with predominant erythroid precursors. Dyserythropoiesis is inevitably present, but the degree of dysplasia is variable in individual cases. Megaloblastoid changes are frequently seen. The cytoplasm of nucleated erythroid cells is usually PAS positive. RS may also be encountered, but they are $<15\%$. Dysplastic changes in granulocytes and megakaryocytes are seldom demonstrated. If the changes are marked, the case should be classified under other categories.

In some cases, the bone marrow may be hypocellular with erythroid hypoplasia resembling aplastic anemia. This condition is more commonly seen in elderly patients. However, the absence of cytopenia in other cell lineage and the increase of dysplastic and immature cells may distinguish hypocellular MDS from aplastic anemia.

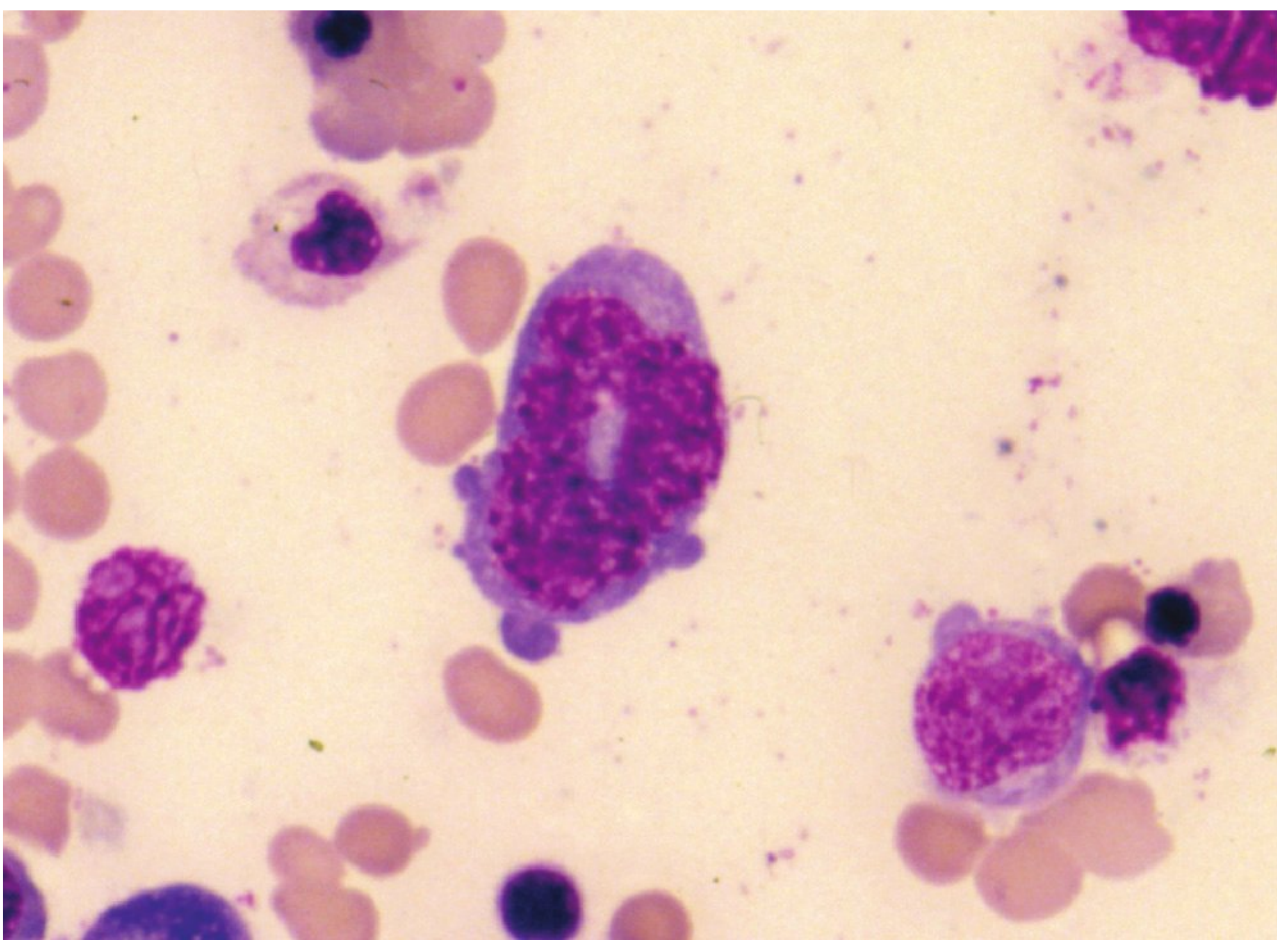


FIGURE 6.3.7 Bone marrow aspirate from a case of MDS, unclassifiable, shows a ring nucleus in a granulocyte. Wright-Giemsa stain, 100× magnification.

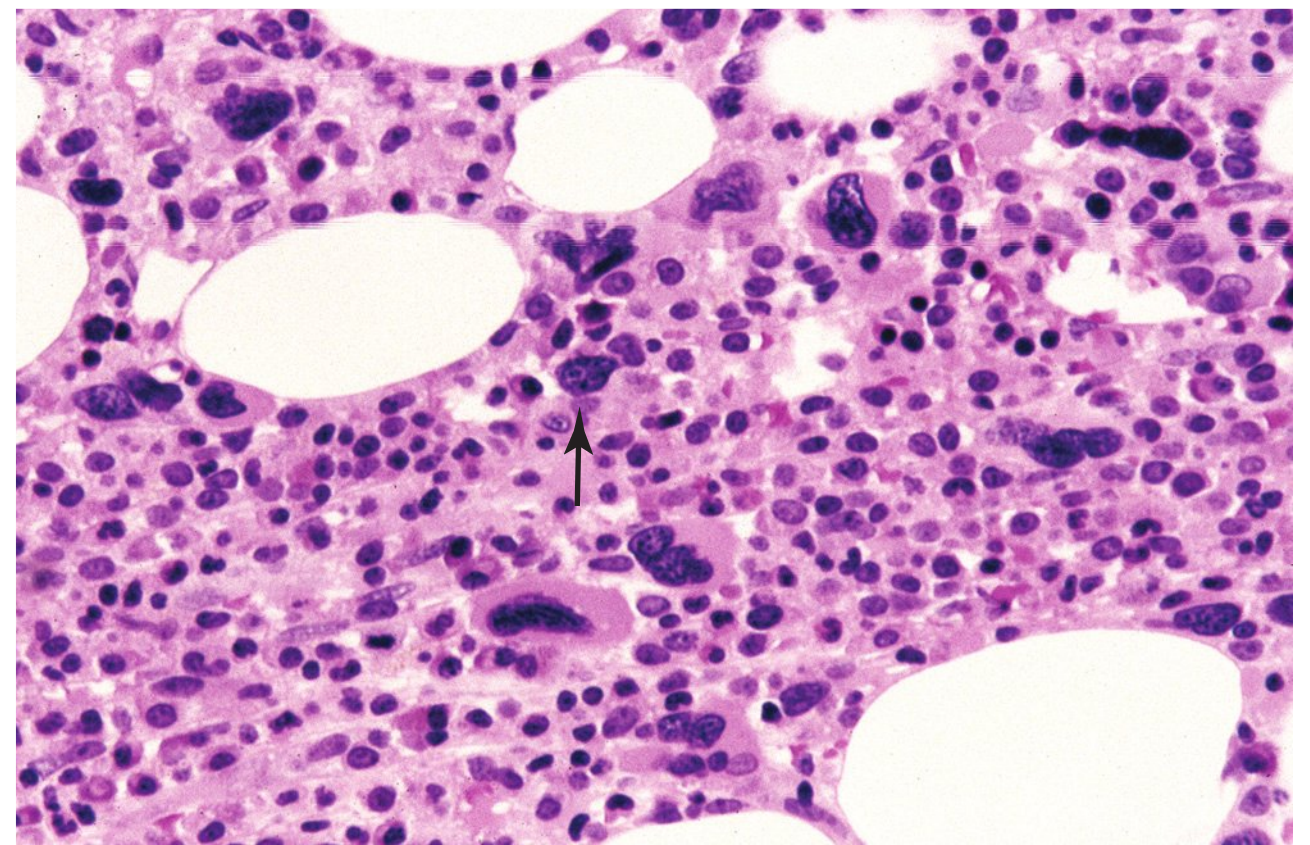


FIGURE 6.3.8 Bone marrow core biopsy from a case of RCMD shows many dysplastic megakaryocytes with microcytic and hypolobated morphology. Naked nuclei (arrow) are also present. Hematoxylin and eosin stain, 100× magnification.

Refractory Neutropenia RN is defined by the presence of $\geq 10\%$ dysplastic neutrophils in the peripheral blood and/or bone marrow, showing mainly nuclear hypolobation and hypogranulation. The other myeloid cell lines should not show significant dysplasia. Causes for secondary neutropenia, such as chemotherapy, radiation therapy, toxins, infections, and autoimmune diseases, should be excluded before the diagnosis of RN is made.

Refractory Thrombocytopenia RT is defined by the presence of $\geq 10\%$ dysplastic megakaryocytes of at least 30 megakaryocytes evaluated. The dysplastic changes include hypolobation, binucleation, multinucleation, and micro-megakaryocytes. The megakaryocytes may be increased or decreased. The other myeloid cell lines should not show

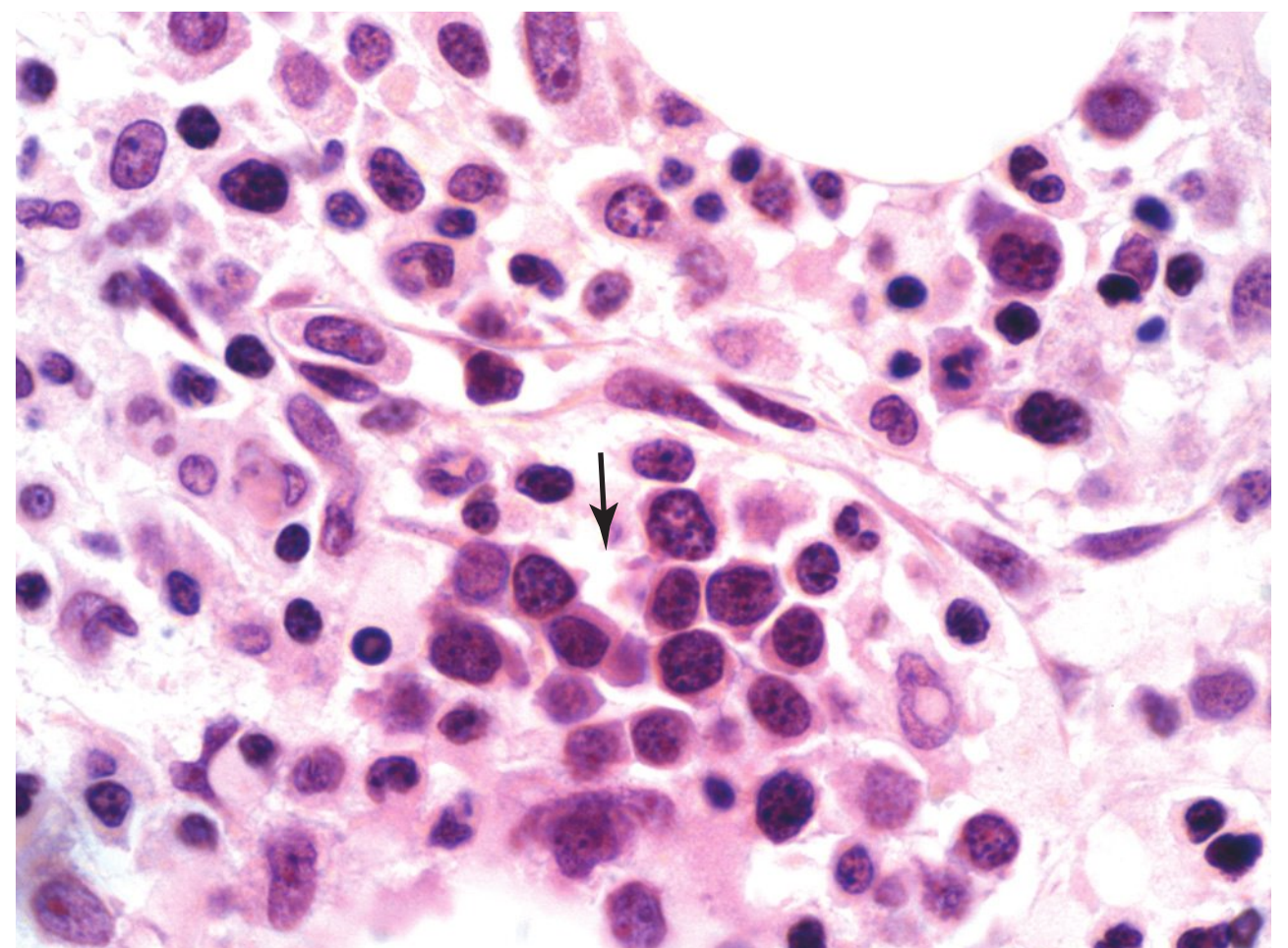


FIGURE 6.3.9 Bone marrow core biopsy from a case of RAEBs shows a cluster of immature myeloid cells (arrow) in between the bony trabeculae representing ALIP. Hematoxylin and eosin stain, 100× magnification.

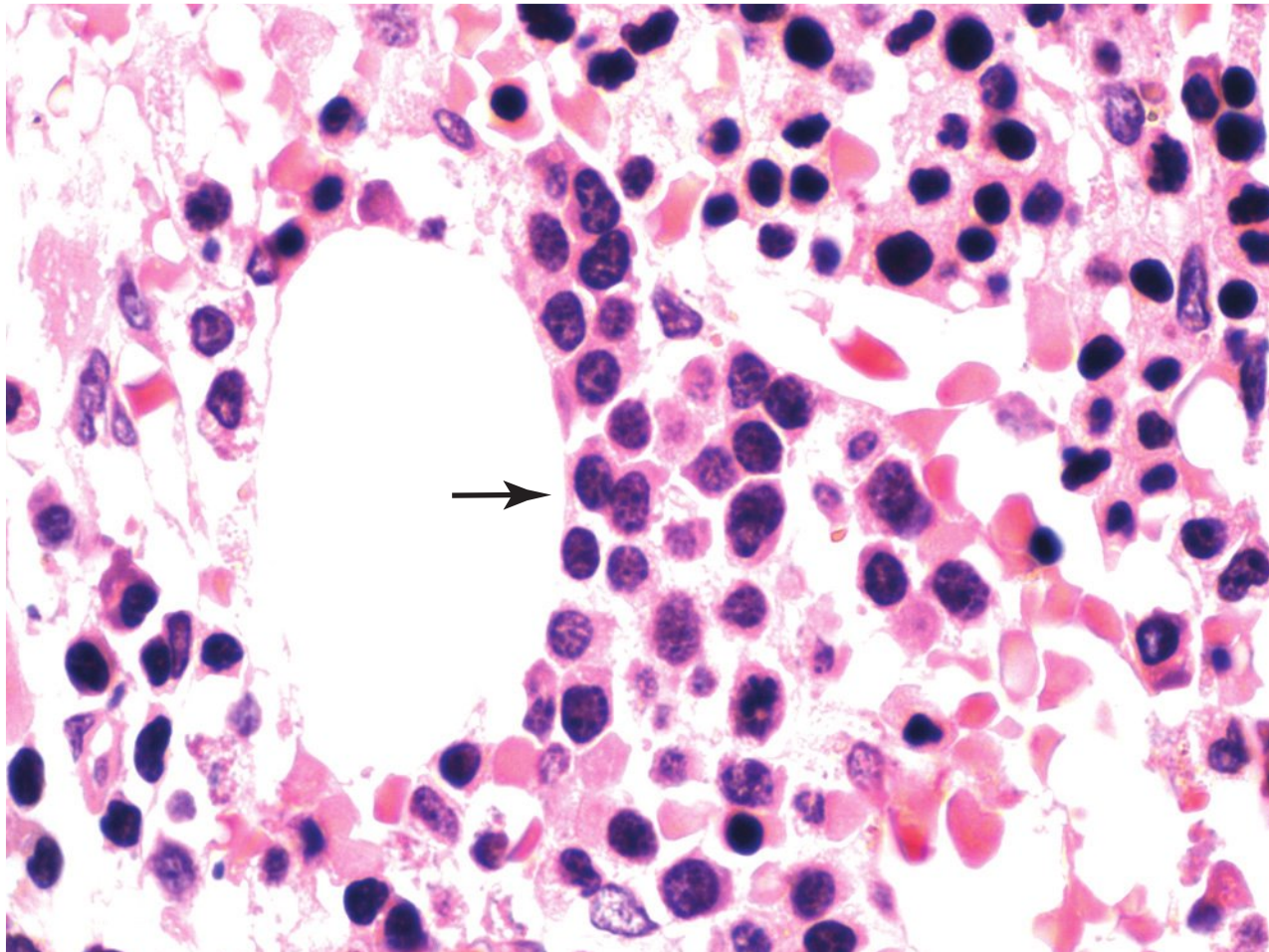


FIGURE 6.3.10 Bone marrow core biopsy from a case of RARSs shows a cluster of pronormoblasts (arrow) representing pseudo-ALIPs. The pronormoblasts have regular nuclear contour and erythroid chromatin pattern. The surrounding mature normoblasts also help to identify the erythroid origin of the immature cells. Hematoxylin and eosin stain, 100× magnification.

significant dysplasia. The distinction of RT from chronic autoimmune thrombocytopenia is important, but it is sometimes difficult for their differentiation.

Refractory Anemia with Ring Sideroblasts

RARS is associated with anemia with dimorphic features; hypochromic and normochromic populations are present in the peripheral blood. The red blood cells can be normocytic or macrocytic. Basophilic stippling, Pappenheimer bodies, and nucleated erythrocytes are more frequently demonstrated in the peripheral blood in RARS than in other forms of MDS. The numbers of granulocytes and platelets are normal in most cases, but they may be decreased in some cases. Blasts, if present in peripheral blood, are <1%. The bone marrow is usually hypercellular with predominance of erythroid series. Dyserythropoiesis with megaloblastoid change is present in variable degrees. The major distinction between RARS and RA is the presence of >15% ring sideroblasts in the bone marrow. Hemosiderin-laden macrophages may be abundant in some cases. Dysplastic changes are absent or mild in myeloid and megakaryocytic series. The number of blasts in the bone marrow is <5%. As in RA, normocellular or hypocellular bone marrow can be demonstrated in rare cases of RARS.

Refractory Anemia with Excess Blasts

RAEB is defined by the presence of 5% to 19% of blasts in the bone marrow and 2–19% of blasts in the peripheral blood. Because the percentages of blasts in the bone marrow and peripheral blood affects the prognosis of patients (18), RAEB is further classified as RAEB-1 when there are 5% to 9% blasts in the bone marrow or 2% to 4% blasts in the blood (4,14). When the bone marrow and blood show 10% to 19% of blasts or Auer rods are seen in the blasts

even when the percentage is <10%, it is classified as RAEB-2. Patients with 5% to 19% blasts in the blood and <10% blasts in the bone marrow are also classified as RAEB-2 (4).

RAEB patients are inevitably anemic. The anemia is normochromic and normocytic or macrocytic. Anisopoikilocytosis is frequently present together with nucleated red blood cells in the peripheral blood. Most patients are pancytopenic with neutropenia and thrombocytopenia. Dysplastic granulocytes and atypical platelets are also present in the peripheral blood.

The bone marrow is hypercellular with panmyeloid hyperplasia. The increase in blasts is usually accompanied by an increase in promyelocytes. Dysplasia can be demonstrated in all cell lineages: myeloid, erythroid, and megakaryocytes. ALIP is frequently present in this category of MDS. Ring sideroblasts may be demonstrated in bone marrow, and in some cases may exceed 15% of the nucleated erythrocytes. Cases that show hypocellular bone marrow with erythroid hypoplasia should be distinguished from aplastic anemia. The presence of dysplastic changes in granulocytes and megakaryocytes and the increase in immature cells as demonstrated by CD34 and CD117 should help to distinguish RAEB from aplastic anemia.

Refractory Cytopenia with Multilineage Dysplasia

RCMD shows bicytopenia or pancytopenia and evidence of multilineage dysplasia, but no increases in blasts or monocytes, and no Auer rods are demonstrated. This type of MDS does not fit into any categories of the FAB classification and is designated by the WHO classification as a new entity. The type and degree of dysplastic changes may vary from patient to patient, and no unifying morphologic feature has been established for this category. The RSs are usually <15% of nucleated erythrocytes. Previously, cases with more than 15% ring sideroblasts were classified as RCMD and RSs (RCMD-RS). This term has been eliminated in the 2008 WHO classification. The bone marrow is hypercellular with bilineage or trilineage hyperplasia, resembling RAEB without an increase in blasts. If blasts are present, they are <5%.

MDS Associated with 5q- Syndrome

When cases of MDS are associated with an isolated del(5q) chromosome abnormality, it is classified as a distinct entity by WHO (4). The clinical significance of identifying this particular karyotype is its association with long survival. Cases in which additional karyotypic abnormalities are found should not be included in this entity. The peripheral blood findings include macrocytic anemia, normal to high platelet count, and normal to slightly elevated leukocyte count. The most characteristic finding is dysmegakaryopoiesis in the bone marrow (19). Megakaryocytes are increased with the presence of micromegakaryocytes or hypolobulated or mononucleated megakaryocytes. Dysplastic granulocytes and increased myeloblasts may be encountered. Erythroid series, in contrast, are hypoplastic. Patients with 5q- syndrome have low frequency of leukemic transformation and relatively long survival.

TABLE 6.3.2

Cytogenetic Abnormalities and Frequency in MDS

Abnormality	MDS	t-MDS
Gain or loss of chromosome material		
+8	10%	
−7 or del(7q)	10%	50%
−5 or del(5q)	10%	40%
del(20q)	5–8%	
−Y	5%	
i(17q) or t(17p)	3–5%	
−13 or del(13q)	3%	
del(11q)	3%	
del(12p) or t(12p)	3%	
del(9q)	1–2%	
idic(X)(q13)	1–2%	
Translocations or inversions		
t(11;16)(q23;p13.3)		3%
t(3;21)(q26.2;q22.1)		2%
t(1;3)(p36.3;q21.2)	1%	
t(2;11)(p21;q23)	1%	
inv(3)(q21q26.2)	1%	
t(6;9)(p23;q34)	1%	

MDS, myelodysplastic syndrome; t-MDS, therapy-related MDS.

Myelodysplastic Syndrome, Unclassifiable

MDS-U is used for cases that do not satisfy the definition of the above categories. The 2008 WHO scheme defines three possible situations that qualify patients for inclusion in this category (8): (a) patients with findings of RCUD or RCMD with 1% blasts in the peripheral blood, (b) cases of MDS with unilineage dysplasia, which are associated with pancytopenia, and (c) patients with persistent cytopenia(s) with 1% or fewer blasts in the blood and fewer than 5% in the bone marrow, unequivocal dysplasia in less than 10% of the cells in one or more myeloid lineages, and who have cytogenetic abnormalities considered as presumptive evidence of MDS (Table 6.3.2).

Childhood Myelodysplastic Syndrome

Primary MDS is very rare in children. Therefore, causes of secondary MDS, such as congenital or acquired bone marrow failure syndromes or therapy-related MDS, should be routinely excluded (8,20). Although many of the morphologic, immunophenotypic, and genetic features seen in MDS in adults are also present in pediatric cases, some MDS subtypes are seldom encountered in childhood form. For instance, RARS and 5q- syndrome are extremely rare

in children. In contrast to adult MDS, in which isolated anemia is a frequent presentation, multilineage dysplasia is more common in childhood MDS (8,20). Pediatric MDS is more often preceded with antecedent events, such as chemotherapy, radiation therapy, and some congenital syndromes (20). Another specific phenomenon in pediatric patients is that some cases with 20% to 29% blasts in the bone marrow and/or blood as well as morphologic and cytogenetic evidences of MDS may behave more like MDS than AML, so that the FAB terminology “RAEB in transformation” is probably more appropriate to describe this condition rather than designating it as AML with myelodysplasia-related changes. In the 2008 WHO classification, a provisional entity, refractory cytopenia of childhood (RCC) is introduced. RCC is similar to RCMD except that in RCC the peripheral blast count is <2% (instead of <1% in RCMD), bone marrow hypocellularity is common, and trilineage dysplasia is significant (8,20).

Immunophenotype

Many flow cytometric studies on MDS have been reported, and most studies claimed that a high percentage of MDS cases showed immunophenotypic abnormalities (21–26). Other studies claimed that immunophenotype is a good prognosticator correlating well with the International Prognostic Scoring System (27,28). However, these abnormalities are variable from case to case, and characteristic immunophenotypes have not yet been established. Furthermore, a large panel of antibodies is usually required, and many of those antibodies are not commonly used. Many abnormalities are based on the intensity of certain markers or on the abnormal location or distribution of a cell cluster as determined by a pair of antibodies. Therefore, further standardization is necessary before reliable immunophenotypes are established. So far, there are no reliable markers to evaluate erythroid and megakaryocytic series. Thus, only selected myelomonocytic markers are discussed in this case.

The immunophenotypes of MDS are usually based on quantitative changes of surface antigens by comparing MDS cases with normal controls. In general, the antigens expressed on normal myeloid precursors (such as CD34, CD117, and HLA-DR) and those on immature granulocytes (such as CD13 and CD33) are increased in MDS (21–24). In contrast, antigens that are expressed on mature granulocytes (such as CD10, CD11b, CD11c, CD16, and CD64) are decreased in MDS (21–24,29,30).

Among all the quantitative changes, CD34 abnormality is most thoroughly studied (22,24,28,31). The percentage of CD34 cells is usually proportional to the number of blasts. Therefore, its percentage increases progressively from RA and RARS to RAEB and leukemic transformation (22). However, not all blasts express CD34, yet immature myeloid cells may show CD34. One study also found that CD34 expression in nonblast myeloid cells was significantly higher in therapy-related MDS than de novo MDS (24). In the study by Xu et al. (24), the percentage of CD34-positive nonblast myeloid cells was $12\% \pm 15\%$ in MDS cases with normal karyotype, but it was $23\% \pm 17\%$ in those with cytogenetic abnormalities (24). Qualitative changes are mainly manifested as aberrant expression of nonmyeloid markers,

namely, T-cell, B-cell, and natural killer-cell markers. CD7 and CD19 can be detected on maturing myeloid cells or monocytes (27). CD56 may be found on myeloblasts (25) and maturing myeloid cells and monocytes (27). Abnormal patterns of CD11b versus CD16 expression or CD13 versus CD16 expression (23,27,32) and abnormal clustering of various cell markers (28) have also been identified in MDS cases. Finally, the low side-scatter property in the nonblastic myeloid cells in MDS cases represents the presence of hypogranular granulocytes (24).

Immunohistochemical staining may help to determine the cell lineage in core biopsy not only in terms of quantity but also in distribution of different cell types. For instance, it may help to distinguish ALIP from pseudo-ALIP.

Myeloid makers that can be used for immunohistochemical stains include myeloperoxidase, lysozyme, elastase, CD15, and CD68 (13,33). Erythrocytes can be identified by hemoglobin A or glycophorins A and C. Megakaryocytes are positive for CD41, CD42b, CD61, and factor VIII. CD34 and proliferating cell nuclear antigen can be used to identify hematopoietic precursors with a strong myeloid commitment, which is particularly useful to identify true ALIP (33). The demonstration of CD34-positive aggregates, particularly large aggregates, is a predictor for poor prognosis regarding both leukemic transformation and survival. CD117 staining may serve the same function as CD34.

In the current case, the patient had pancytopenia with macrocytic anemia. The bone marrow was hypercellular showing dysplastic changes in erythroid and megakaryocytic cell lines and 12% myeloblasts. The flow cytometry revealed high percentages of CD13, CD33, CD34, and CD117. The constellation of the clinicopathology findings is characteristic of RAEB-2. The patient responded well with erythropoietin and G-CSF and was discharged from the hospital promptly. However, the presence of high percentages of CD34 and CD117 in this patient is associated with an unfavorable prognosis.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry may identify quantitative and qualitative abnormalities in MDS cases, even though they are nonspecific and cannot be depended upon for a definitive diagnosis. The most useful role of flow cytometry in MDS cases is the identification of myeloblast. The basic phenotype of myeloblast is CD45^{dim} SSC^{low} CD34⁺ CD117⁺ (26). However, the use of flow cytometry to determine the blasts should be interpreted with caution, because hemodilution or destruction of nucleated erythrocytes in the process of red cell lysis may render the count inaccurate (20). In addition, CD34 and CD117 can be present in cells other than myeloblasts. Nevertheless, the aberrant myeloblasts may show a variety of changes. The first European LeukemiaNet working conference in flow cytometry in MDS regards the following features most relevant for the identification of abnormal blasts: an increased percentage of myeloblasts; abnormal granularity (SSC); abnormal intensity of CD45, CD34, or CD117, or expression of CD11b or CD15; abnormal intensity (or lack) of HLA-DR; and the expression of lineage infidelity markers, such as TdT, CD7, CD19, or CD56 (26).

Immunohistochemistry, in contrast, is used to demonstrate the distribution of various cell types and is helpful to identify ALIP. CD34 and CD117 staining can help to enumerate myeloblasts in tissue. CD42b and CD61 are useful for identification of micromegakaryocytes in histologic sections.

Molecular Genetics

The basic phenomenon in MDS is ineffective hematopoiesis. Therefore, it is important to find out the mechanisms that induce such a phenomenon. The most popular hypothesis is an increase of apoptosis in the early phase of MDS, as supported by histochemical, flow cytometric, and biochemical studies (2,34–37). A flow cytometric study, using Annexin V to enumerate apoptotic CD34 cells and Ki-67 to enumerate proliferative cells, found that apoptosis was significantly increased in RA, RARS, and RAEB cases (38). However, in RA and RARS, apoptosis always exceeded proliferation, whereas in RAEB, apoptosis was equalized with proliferation. The same study also found that the proapoptotic (Bax/Bad) versus antiapoptotic (Bcl-2/Bcl-x) protein ratio was increased in RA/RARS, whereas disease progression was associated with significantly reduced ratio.

Apoptosis may be induced by cytokines such as tumor necrosis factor- α and interferon- γ (34,35). Cell culture studies showed that these cytokines can suppress the growth of hematopoietic progenitors and induce Fas expression on CD34 cells (35). The increase of these cytokines and Fas expression has been demonstrated in some MDS patients. These studies implicate the potential role of the Fas/Fas ligand system in the induction of apoptosis. In clinical practice, the inhibition of apoptosis with hematopoietic growth factors and erythropoietin may benefit in improving the blood counts.

MDS has proved to be a clonal disease by molecular genetic techniques. Cytogenetic abnormalities are frequently demonstrated in MDS patients, approximately 30% to 50% in primary MDS cases and in >80% of therapy-related MDS cases (39). Most of the abnormalities are numerical, such as chromosome 5 and 7 monosomy and deletion of the long arm of chromosomes 5 and 7 (36,39,40). Structural aberrations, such as inversion of chromosome 3 and translocations also occur. Translocations include translocation-Ets-leukemia (TEL) fusion, mixed-lineage leukemia fusion, nucleoporin abnormality, ecotropic viral integration/site (EVI-1) family expression, and others (36). Recurrent translocations also occur involving the MDS1-EVI1 locus on chromosome 3q and between partners on chromosomes 6p and 9q (41). Other genes that are involved in rare translocations include RUNX1, TEL, MEL1, NUP98, and IE3 (41). Point mutations are also encountered in several genes. TET2 mutation is most frequent (>20%) in MDS, followed by RUNX1, NRAS/KRAS, ASXL1, TP53 (about 10% to 20%). The least frequently involved genes include CBL and JAK2 (41). While RUNX1, NRAS/KRAS and TP53 confer a poor prognosis, TET2 and JAK2 are associated with favorable outcome (41).

The International MDS Risk Analysis Workshop divided MDS cases into three prognostic cytogenetic groups (18). Good

outcomes were those with normal chromosomes, -Y alone, del(5q) alone, and del(20q) alone. Poor outcomes were associated with complex abnormalities (more than three abnormalities) or chromosome 7 anomalies. Intermediate outcomes were associated with cytogenetic aberrations besides those mentioned above. There are a few chromosome abnormalities that are associated with relatively well-defined morphologic clinical syndromes (14,25).

5q- Syndrome

As mentioned above, 5q-syndrome is designated as a separate entity in the WHO classification of MDS. However, 5q-, either isolated or associated with other cytogenetic abnormalities, is seen in a large variety of hematologic disorders, especially myeloid diseases (19). As a sole abnormality, 5q- syndrome is most frequently seen in MDS and AML, particularly in therapy-induced cases. It is predominantly (68% to 74%) seen in female patients. In MDS cases, 5q-syndrome may be seen in RA, RAEB, and RCMD.

Monosomy 7 Syndrome of Childhood

This syndrome is seen in children aged 6 months to 8 years. It is male predominant. The peripheral blood may show anemia, thrombocytopenia, monocytosis, and leukoerythroblastosis. The bone marrow may present with dysplastic changes in erythroid, granulocytic, and monocytic series. Megakaryocytes are usually normal morphologically but may be decreased in >50% of patients. Myeloblasts may be present in the peripheral blood, but they are usually <2%. In the bone marrow, the range of blasts is between 3% and 11%. Because neutrophils have defective chemotaxis, patients may have recurrent infections. The syndrome shares many clinical and hematologic features with juvenile myelomonocytic leukemia, and their distinction may not be possible in some cases. This syndrome is usually associated with a poor prognosis.

del(17p)

This anomaly is associated with a particular type of dysgranulopoiesis characterized by small neutrophils with pseudo-Pelger-Huet nuclei and cytoplasmic vacuoles. Some mature neutrophils show completely nonlobulated nuclei. These patients usually have a high incidence of p53 mutation and an unfavorable prognosis.

inv(3)(q21;q26.2) or t(3;3)(q21;q26.2)

Patients with this abnormality usually have normal or increased platelet counts in the peripheral blood and dysmegakaryocytosis in the bone marrow. The megakaryocytes are increased, showing micromegakaryocytes with hypolobated nuclei. This anomaly may be seen in RARS or RAEB, and is generally associated with an unfavorable outcome.

Dysregulated Genes in MDS

The pathogenesis of MDS is highly complicated as it involves many dysregulated genes, which include many oncogenes, cell-cycle regulatory genes, apoptotic genes, angiogenic genes, genes regulating DNA methylation,

TABLE 6.3.3

Salient Features for Laboratory Diagnosis of MDS

1. Peripheral cytopenia in 1–3 cell lineages
2. Dysplasia in 1–3 cell lineages demonstrated in peripheral blood and bone marrow
3. Usually hypercellular bone marrow
4. Increased ring sideroblasts
5. Increases in myeloid precursor (CD34, CD117, HLA-DR) and immature granulocytic (CD13, CD33) antigens
6. Decreases in mature granulocytic antigens (CD10, CD11b, CD11c, CD16, CD64)
7. Aberrant expression of nonmyeloid markers (CD7, CD19, CD56)
8. Identification of cell lineage and distribution by immunohistochemistry
9. Cytogenetic abnormalities: see Table 6.3.2

genes regulating histone acetylation, receptor tyrosine kinase genes, and immunomodulatory genes. A detailed description of the involvement of these genes in MDS can be found in reviews by Nishino and Chang (37) and Bejar and Ebert (41).

A complete list of recurring chromosome abnormalities and their frequency in MDS is cited from the 2008 WHO classification (Table 6.3.2). The presence of +8, -Y or del(20q) in the absence of morphologic evidence is not considered diagnostic for MDS. However, the presence of the remaining abnormalities in the setting of persistent cytopenias is considered presumptive evidence of MDS even in the absence of definitive morphologic features (8).

Patterns of gene expression are commonly altered in MDS not only due to genetic mutations but also due to epigenetic chromosome modifications. The most common epigenetic change in MDS is DNA methylation (41,42). Several genes are transcriptionally silenced in association with promoter DNA methylation. These include genes involved in cell-cycle regulation, (CDKN2A), apoptosis (DAPK1 and RIL), adhesion and motility (CDH1 and CDH13), and other pathways (42).

The salient features for laboratory diagnosis of MDS are summarized in Table 6.3.3.

Clinical Manifestations

Primary MDS is usually seen in patients >50 years (14). The incidence of MDS increases dramatically after 40 years of age and rises to >20 cases per 100,000 people aged 70 years and older (1). However, it may also be seen in children, particularly those with specific cytogenetic abnormalities, such as monosomy 7 syndrome of childhood. Secondary MDS is usually seen in elderly persons with exposure to radiation or chemotherapy. Patients with secondary MDS fare poorer with more rapidly progressive marrow failure than do those with primary MDS.

TABLE 6.3.4

International Prognostic Scoring System for MDS

Score	0	0.5	1.0	1.5	2.0
% Blasts	<5	5–10	—	11–20	20–30*
Karyotype [†]	Good	Intermediate	Poor	—	—
Cytopenias [‡]	0–1	2–3	—	—	—

*Current WHO range of acute myeloid leukemia.

[†]See text.

[‡]Cytopenias are defined as Hb <10 g/dL, neutrophils <1,500/mL, and platelets <100,000/μL. MDS, myelodysplastic syndrome.

Clinical symptoms are related to cytopenia. For instance, dyspnea and pallor are due to anemia; fever, oral pain (agranulocytic angina), and recurrent bacterial or fungal infections are associated with neutropenia; and ecchymoses, petechiae, and mucocutaneous bleeding are related to thrombocytopenia (1). At the end stage, the patient may progress to complete bone marrow failure or leukemic transformation. RA and RARS have a low incidence of leukemic transformation, whereas RAEB has a higher incidence of transformation and rapidly progressive marrow failure. The clinical condition of RCMD is intermediate between these two groups (14).

According to the International MDS Risk Analysis Workshop, the prognosis of MDS depends on cytogenetic abnormalities, percentage of myeloblasts in the bone marrow, and the number of cytopenia (Table 6.3.4) (18). Age and gender also affect survival. Patients >60 years and men have shorter survival.

REFERENCES

1. Rothstein G. Disordered hematopoiesis and myelodysplasia in the elderly. *J Am Geriatr Soc.* 2003;51(suppl):S22–S26.
2. Dansey R. Myelodysplasia. *Curr Opin Oncol.* 2000;12:13–21.
3. Bennett J, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol.* 1982;51:189–190.
4. Brunning RD, Bennett JM, Flandrin G, et al. Myelodysplastic syndromes. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:61–74.
5. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood.* 2002;100:2292–2302.
6. Bowen D, Culligan D, Jowitt S, et al. Guidelines for the diagnosis and therapy of adult myelodysplastic syndromes. *Br J Haematol.* 2003;120:187–200.
7. List AF, Vardiman J, Issa JP, DeWitte TM. Myelodysplastic syndromes. *Hematology Am Soc Hematol Educ Program.* 2004:297–317.
8. Brunning RD, Orazi A, Germing U, et al. Myelodysplastic syndromes/neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed. Lyon, France: IARC Press; 2008:88–107.
9. Goasguen JE, Bennett JM. Classification and morphologic features of the myelodysplastic syndromes. *Semin Oncol.* 1992;19:4–13.
10. Mufti GJ, Bennett JM, Goasguen J, et al. Diagnosis and classification of myelodysplastic syndrome: International working group on morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. *Haematologica.* 2008;93:1712–1717.
11. Ho PJ, Gibson J, Vincent P, et al. The myelodysplastic syndromes: diagnostic criteria and laboratory evaluation. *Pathology.* 1993;25:297–304.
12. Farhi DC. Myelodysplastic syndromes and acute myeloid leukemia: diagnostic criteria and pitfalls. *Pathol Annu.* 1995;30(pt 1):29–57.
13. Rosati S, Anastasi J, Vardiman J. Recurring diagnostic problems in the pathology of myelodysplastic syndrome. *Semin Hematol.* 1996;33:111–126.
14. Brunning RD. Myelodysplastic syndromes. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1717–1743.
15. Tricot G, DeWolf-Peeters C, Hendricks B, et al. Bone marrow histology in myelodysplastic syndromes. I. Histological findings in myelodysplastic syndromes and comparison with bone marrow smears. *Br J Haematol.* 1984;57:423–430.
16. Tricot G, DeWolf-Peeters C, Vlietinck R, et al. Bone marrow histology in myelodysplastic syndromes. II. Prognostic value of abnormal localization of immature precursors in MDS. *Br J Haematol.* 1984;58:217–225.
17. Kraus MD, Bartlett NL, Fleming MD, et al. Splenic pathology in myelodysplasia: a report of 13 cases with clinical correlation. *Am J Surg Pathol.* 1998;22:1255–1266.
18. Greenberg P, Cox C, LeBeau MM, et al. International Scoring System for evaluating prognosis in myelodysplastic syndromes. *Blood.* 1997;89:2079–2088.
19. Ven Den Berghe H, Michaux L. 5q-, twenty-five years later. A synopsis. *Cancer Genet Cytogenet.* 1997;94:1–7.
20. Head DR, Hamilton KS. The myelodysplastic syndromes. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology.* Philadelphia, PA: Elsevier; 2011; 656–671.
21. Dunphy CH. Applications of flow cytometry to chronic myeloproliferative disorders and myelodysplastic syndromes. *J Clin Ligand Assay.* 2004;27:170–179.
22. Orfao A, Ortuno F, de Santiago M, et al. Immunophenotyping of acute leukemias and myelodysplastic syndromes. *Cytometry A.* 2004;58:62–71.

23. Statler-Stevenson M, Arthur DC, Jabbour N, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. *Blood*. 2001;98:979–987.
24. Xu D, Schultz C, Akker Y, et al. Evidence for expression of early myeloid antigens in mature, non-blast myeloid cells in myelodysplasia. *Am J Hematol*. 2003;74:9–16.
25. Kussick SJ, Wood BL. Using 4-color flow cytometry to identify abnormal myeloid populations. *Arch Pathol Lab Med*. 2003;127:1140–1147.
26. van de Loosdrecht AA, Alhan C, Bene MC, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. *Haematologica*. 2009;94:1124–1134.
27. Wells DA, Benesch M, Loken MR, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. *Blood*. 2003;102:394–403.
28. Maynadie M, Picard F, Husson B, et al. Immunophenotypic clustering of myelodysplastic syndromes. *Blood*. 2002;100:2349–2356.
29. Shao Z, Shang H, Chen G, et al. Expression and function of c-kit receptor in bone marrow mononuclear cells of patients with myelodysplastic syndromes. *Chin Med J*. 2001;114:481–485.
30. Chang CC, Cleveland RP. Decreased CD10-positive mature granulocytes in bone marrow from patients with myelodysplastic syndrome. *Arch Pathol Lab Med*. 2000;124:1152–1156.
31. Sawada K, Sato N, Notoya A, et al. Proliferation and differentiation of myelodysplastic CD34+ cells: phenotypic subpopulations of marrow CD34+ cells. *Blood*. 1995;85:194–202.
32. Bowen KL, Davis BH. Abnormal patterns of expression of CD16 (FcR-III) and CD11b (CRIII) antigens by developing neutrophils in the bone marrow of patients with myelodysplastic syndrome. *Lab Hematol*. 1997;3:292–298.
33. Deliliers GL, Annaloro C, Soligo D, et al. The diagnostic and prognostic value of bone marrow immunostaining in myelodysplastic syndromes. *Leuk Lymphoma*. 1998;28:231–239.
34. Yoshida Y, Mufti GJ. Apoptosis and its significance in MDS: controversies revisited. *Leuk Res*. 1999;23:777–785.
35. Rosenfeld C, List A. A hypothesis for the pathogenesis of myelodysplastic syndromes: implications for new therapies. *Leukemia*. 2000;14:2–8.
36. Hirai H. Molecular mechanisms of myelodysplastic syndrome. *Jpn J Clin Oncol*. 2003;33:153–160.
37. Nishino HT, Chang CC. Myelodysplastic syndromes: clinicopathologic features, pathobiology, and molecular pathogenesis. *Arch Pathol Lab Med*. 2005;129:1299–1310.
38. Parker JE, Nulti GJ, Rasool F, et al. The role of apoptosis, proliferation and the Bcl-2 related proteins in the myelodysplastic syndromes and acute myeloid leukemia secondary to MDS. *Blood*. 2000;96:3932–3938.
39. Willman CL. Molecular genetic features of myelodysplastic syndromes. *Leukemia*. 1998;12(suppl 1):S2–S6.
40. Crisan D. Molecular mechanisms in myelodysplastic syndromes and implications for evolution to acute leukemias. *Clin Lab Med*. 2000;20:49–69.
41. Bejar R, ebert BL. The genetic basis of myelodysplastic syndromes. *Hematol Oncol Clin N Am*. 2010;24:295–315.
42. Issa JP. Epigenetic changes in the myelodysplastic syndrome. *Hematol Oncol Clin N Am*. 2010;24:317–330.

CASE 4

Myelodysplastic/Myeloproliferative Neoplasms

CASE HISTORY

A 63-year-old man presented with a 2-year history of leukocytosis and a 9-kg weight loss during a period of 4 months prior to admission. He saw a hematologist 2 years previously because of elevated leukocyte count, but no conclusive diagnosis was made. The patient did not have a history of exposure to ionizing irradiation or cytotoxic agents. He also denied any fevers, chills, or night sweats. There were no localized symptoms or somatic complaints. Physical examination revealed no splenomegaly or lymphadenopathy.

Hematologic workup revealed a hematocrit of 44%, as compared with 48% recorded 4 months previously. His total leukocyte count was 15,400/ μ L with 64% neutrophils, 21.9% lymphocytes, 8.1% monocytes, 6.0% eosinophils, and 0.4% basophils. The platelet count was 338,000/mL.

Examination of bone marrow aspirate showed a marked increase in monocytic series (10.25%), which

included monocytes, promonocytes, and monoblasts. The total blast count including myeloblasts and monoblasts was 3.8%. There was bone marrow eosinophilia (7.2%) with various developmental stages, up to myelocytes. Dysplastic changes were present within the granulocytic population, such as hypolobation, pseudo-Pulger-Huet cells, and hypogranularity. Megaloblastoid changes were seen frequently in the normoblast population. The core biopsy revealed a myeloid-erythroid (M/E) ratio of 6:1 with predominance of myelomonocytic cells. A few clusters of immature myelomonocytic precursors were present in the intertrabecular areas. Eosinophilia was also demonstrated.

FLOW CYTOMETRIC FINDINGS

Results in the bone marrow were as follows: CD13-CD33, 82%; CD14, 34%; CD34, 12%; HLA-DR, 31%; CD45, 100% (Fig. 6.4.1).

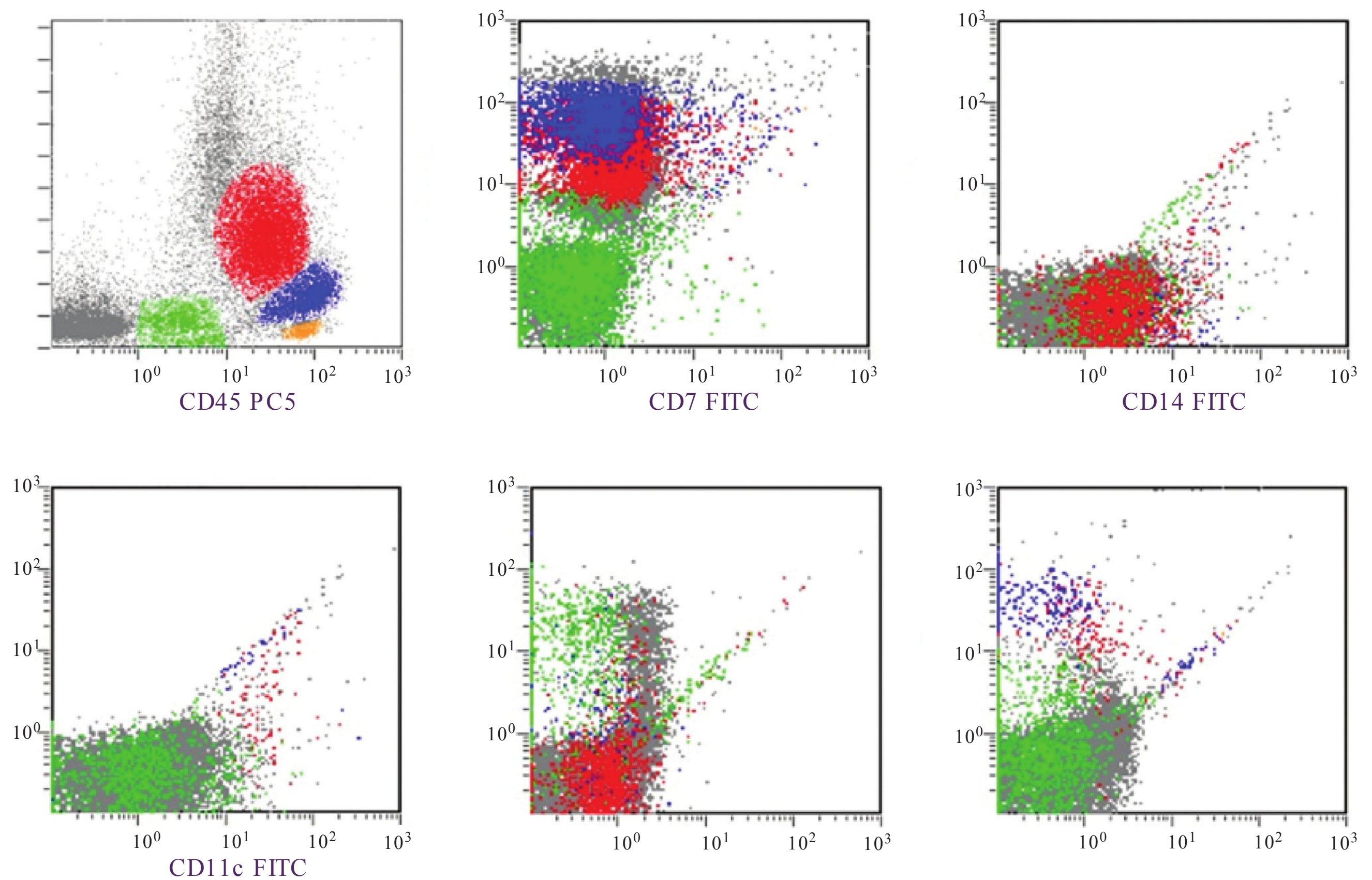


FIGURE 6.4.1 Flow cytometric histograms show three populations: the blue and red populations are mature and immature monocytes, and the green population represents immature myelocytes. The monocyte populations are positive for CD13.33, CD14, CD117, and CD34. The myelocytes are positive for CD13.33, CD117, and CD34. Both populations are negative for CD11c. SS, side scatter; PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; RD1, rhodamine.

IMMUNOHISTOCHEMISTRY AND CYTOCHEMISTRY

Immunohistochemical stain for CD68 (PG-M1) and a-naphthyl butyrate esterase stain highlighted the large population of monocytic series in bone marrow biopsy and bone marrow aspirate, respectively.

MOLECULAR GENETIC FINDINGS

Cytogenetic study demonstrated a 46,XY karyotype, and fluorescence in situ hybridization study showed no rearrangement of the TEL (ETV6) locus associated with a translocation t(5;12).

DISCUSSION

As discussed in Cases 2 and 3, myeloproliferative neoplasms (MPNs) and myelodysplastic syndrome (MDS) can be demonstrated separately in the myeloid series. However, there are cases that present with features of both MPN and MDS. For instance, a study of >500 MDS patients according to

the French-American-British (FAB) scheme revealed that 4.4% of cases had features of both MDS and MPN (1). This is a dilemma for hematologists in terms of management of these patients. Recently, the World Health Organization (WHO) classification created a new entity: myelodysplastic/myeloproliferative diseases (MDS/MPN) that include diseases presenting with both MDS and MPN features (2–6). This new entity allows clinicians to have more flexibility in treating patients according to the predominant feature. For instance, chronic myelomonocytic leukemia (CMML) was previously classified as MDS in the Revised European–American Classification of Lymphoid Neoplasms (REAL classification) (7), but is now included in this new entity and can be treated as MDS or MPN according to its major clinical features.

Historically, the FAB was the first group to recognize the existence of such diseases and in 1994 classified them under the name of chronic myeloid leukemias (CMLs), including chronic granulocytic leukemia, atypical chronic myeloid leukemia (aCML), and CMML (8). The WHO scheme separates the Philadelphia chromosome–positive CML from aCML and CMML. The latter two entities are consistently Philadelphia chromosome–negative, and are designated as MDS/MPN. In addition, the WHO classification includes juvenile myelomonocytic leukemia (JMML) in

TABLE 6.4.1

Salient Features for Laboratory Diagnosis of Chronic Myelomonocytic Leukemia

1. Persistent peripheral monocytosis ($>1,000/\mu\text{L}$) for >3 mo
2. Presence of immature myeloid cells ($<10\%$) in peripheral blood
3. Bone marrow monocytosis ($>10\%$)
4. Myelodysplasia ≥ 1 myeloid lineages
5. No Philadelphia chromosome or BCR-ABL1 fusion gene, but cytogenetic abnormality is present in 20–30% of cases
6. No rearrangement of PDGFRA or PDGFRB
7. Fewer than 20% blasts (myeloblasts, monoblasts, and promonocytes included) in blood and bone marrow

the MDS/MPN category. Refractory anemia with ring sideroblasts (RARS) associated with marked thrombocytosis (RARS-T) is listed as a provisional entity because its nature is unclear (6).

Morphology

The general feature of MDS/MPD is proliferation of one or more of the myeloid lineages in the bone marrow (2,9). This proliferation may produce increased numbers of circulating cells in one or more cell lineages. On the other hand, one or more of the other lineages may be dysplastic, leading to ineffective hematopoiesis in the peripheral blood, such as anemia, leukopenia, or thrombocytopenia. The blast count in the peripheral blood and bone marrow should be $<20\%$. Splenomegaly and hepatomegaly are common.

In this category, CMML is the most common disorder, accounting for 3/100,000 persons annually (2,3,9,10). It is characterized by a substantial monocytosis in both the peripheral blood and the bone marrow (Table 6.4.1). The peripheral blood monocyte count should be $>1000/\mu\text{L}$ (Fig. 6.4.2). When the total leukocyte count is extremely high in other MPNs, the peripheral monocyte count may be higher than 1,000/mL; therefore, the cutoff of 10% monocyte count is more reliable for differential diagnosis under this condition (11,12). The monocyte count in bone marrow is not defined in the WHO classification, but it should be $>10\%$ in the so-called marrow predominant CMML when peripheral monocyte count is $<1,000/\text{mL}$ (Figs. 6.4.3 and 6.4.4) (11). Immature myeloid cells (myelocytes and metamyelocytes) can be present in the peripheral blood, but they are usually $<10\%$. Unlike CML, CMML shows myelodysplastic changes in one or more myeloid lineages, but the Philadelphia chromosome or BCR-ABL1 fusion is not present.

If myelodysplasia is absent or minimal, the diagnosis of CMML can still be made when peripheral monocytosis has lasted for >3 months, other causes of monocytosis have been excluded, and clonal cytogenetic abnormality is present in bone marrow cells (2).

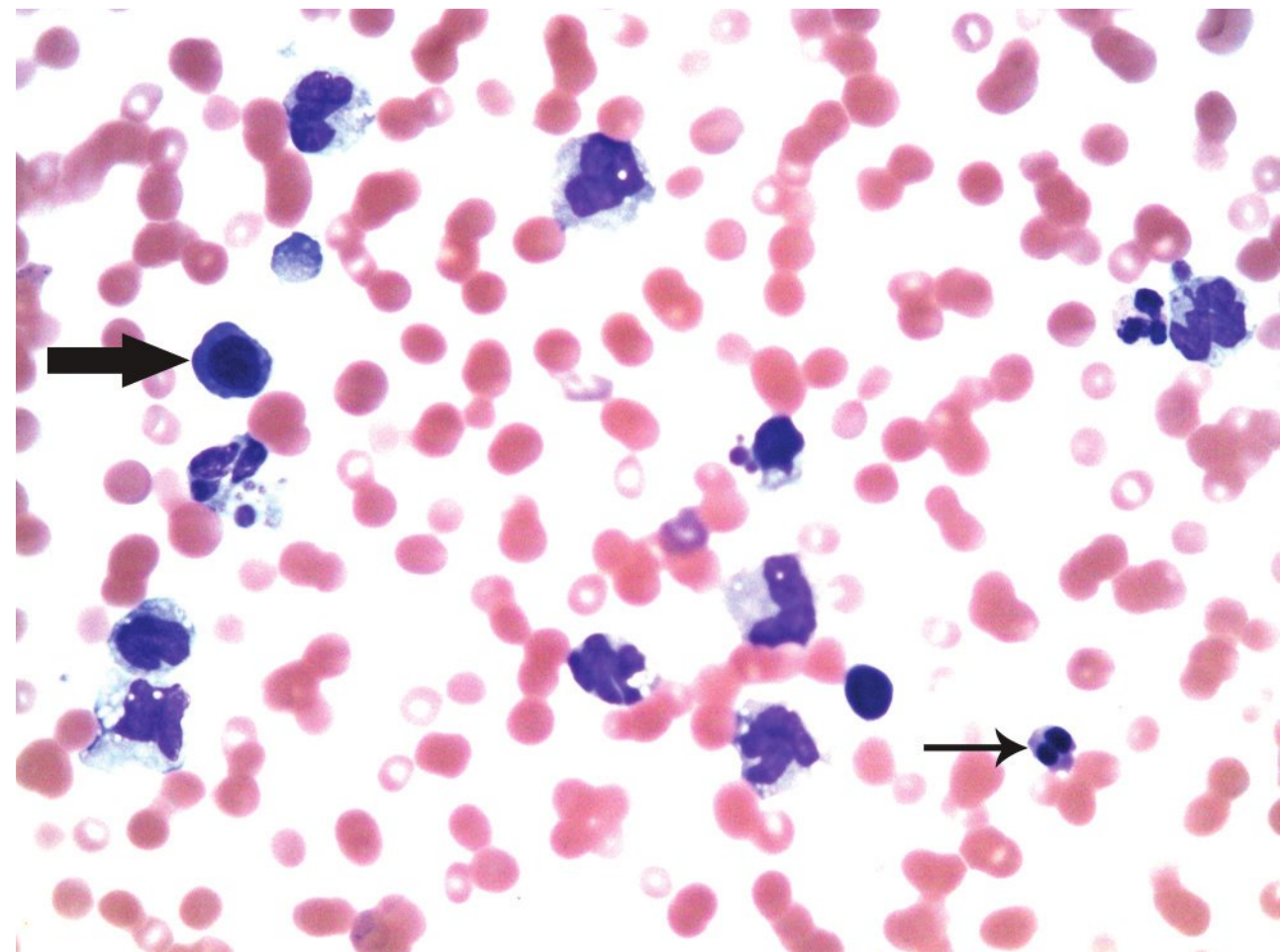


FIGURE 6.4.2 Peripheral blood smear from a case of CMML-2 shows monocytosis with the presence of a blast (large arrow) and a dysplastic nucleated erythrocyte (small arrow). Wright–Giemsa, 60× magnification.

The usefulness of bone marrow core biopsy in the diagnosis of CMML has been emphasized in some recent studies (13–15). The important findings in core biopsy include hypercellularity, predominance of myelomonocytic cells, megakaryocytic dysplasia, and abnormal localization of immature precursors (14).

Unlike acute myeloid leukemia, CMML has $<20\%$ blasts in the blood or bone marrow. If the blasts in the blood are 5% to 19% or 10% to 19% in the bone marrow, it is classified as CMML-2, whereas cases with $<5\%$ blasts in the blood and $<10\%$ in the bone marrow are classified as CMML-1. The M/E ratio in CMML is lower than that in CML and aCML, as there are $>15\%$ of erythroid components in the bone marrow (10). Mild basophilia and mild eosinophilia can be seen in some cases. If eosinophilia is striking ($>1,500/\text{mL}$), it becomes the variant of CMML with eosinophilia, which

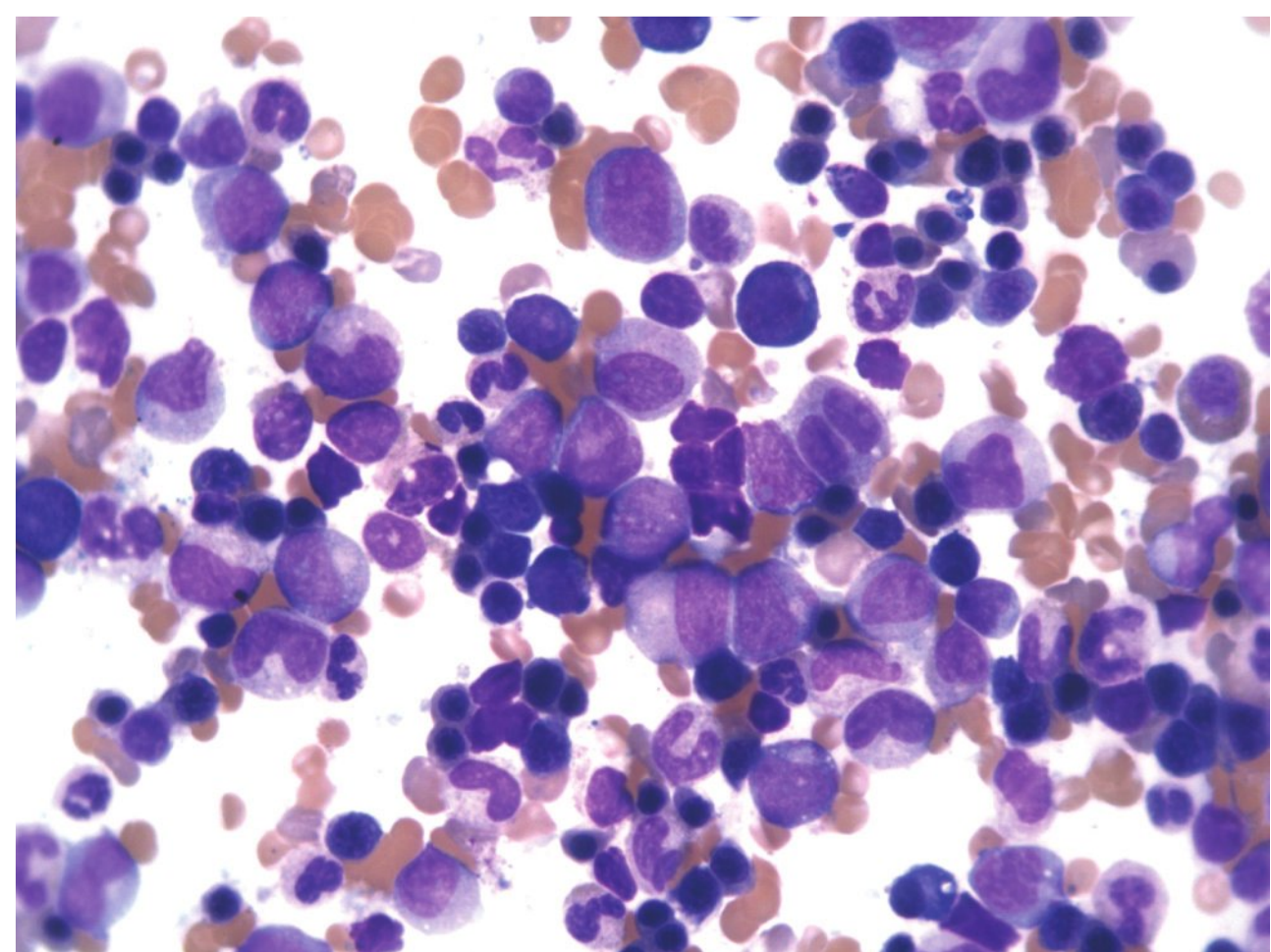


FIGURE 6.4.3 Bone marrow aspirate from a case of CMML-2 shows many mature and immature monocytes. Wright–Giemsa, 60× magnification.

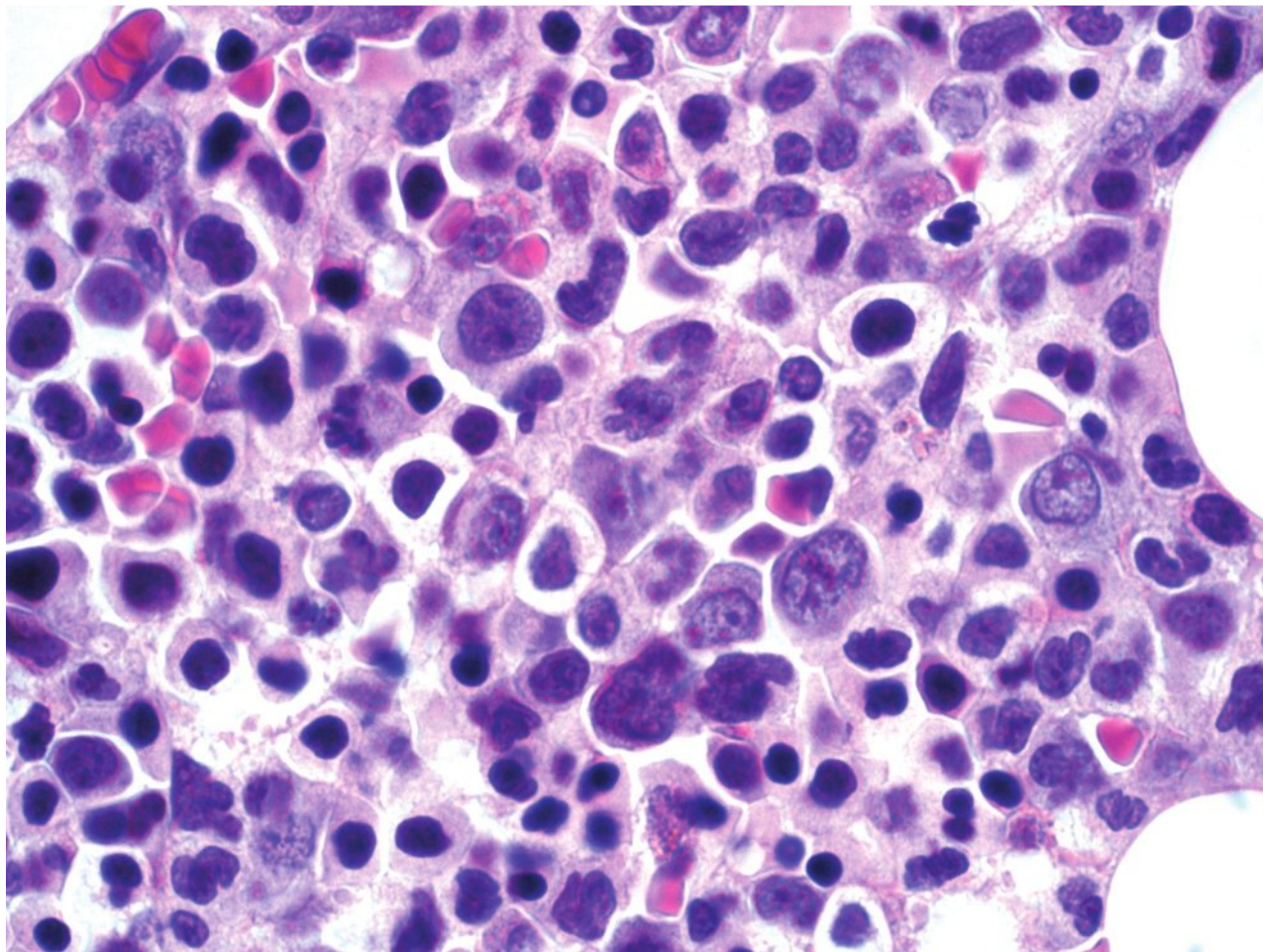


FIGURE 6.4.4 Bone marrow core biopsy shows many large immature monocytes with convoluted nuclei in some cells and mature monocytes. Hematoxylin and eosin, 100× magnification.

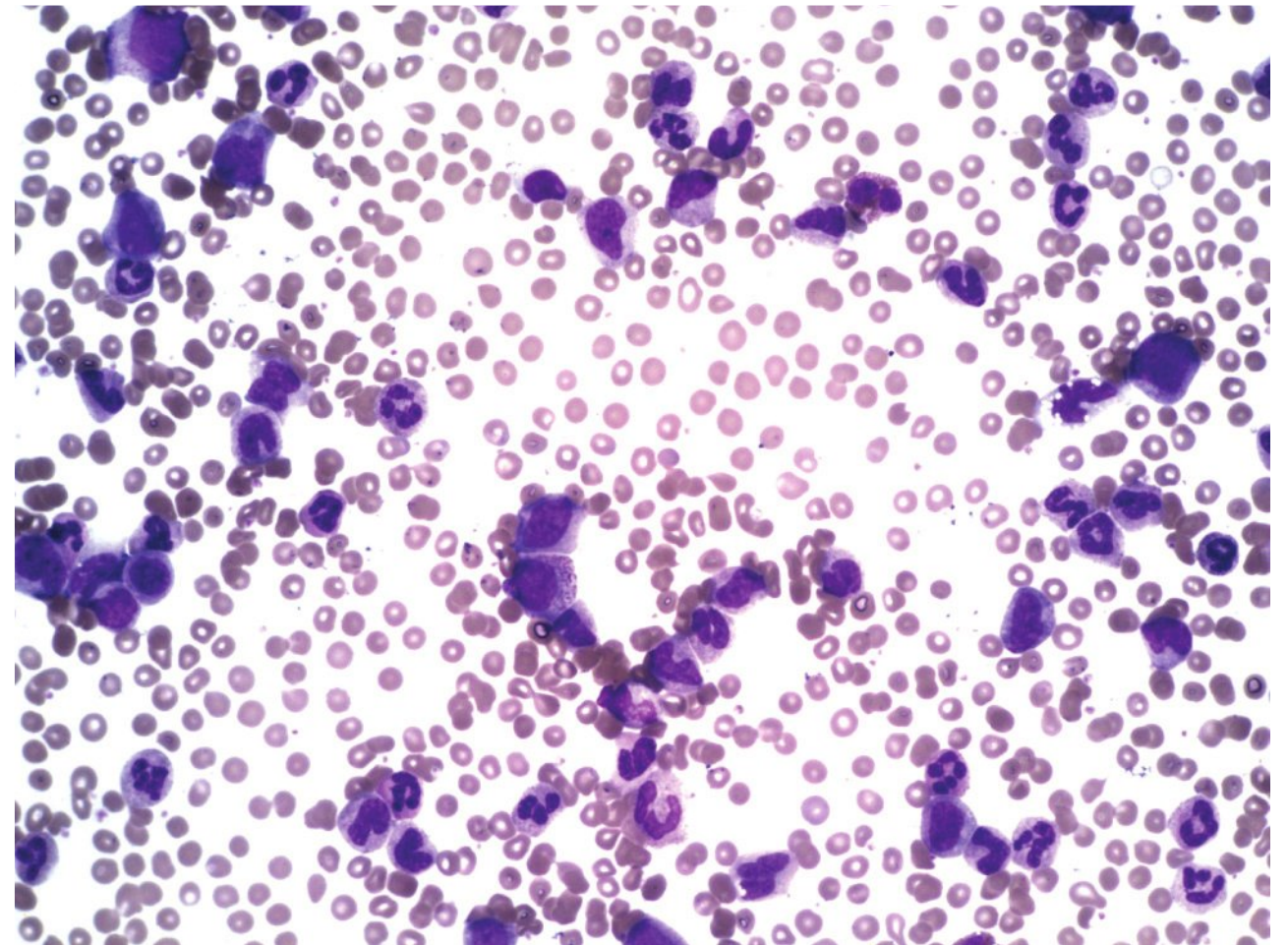


FIGURE 6.4.5 Peripheral blood smear from a case of aCML shows marked leukocytosis with several immature forms. Wright–Giemsa, 40× magnification.

is associated with specific symptoms due to eosinophilic degranulation and may be associated with a specific karyotype, t(5;12) (16,17). The spleen and lymph node can be infiltrated by the myelomonocytic cells. Some patients may have generalized lymphadenopathy. These lymph nodes are characterized by extensive infiltration by plasmacytoid monocytes (18–21). The plasmacytoid monocytes have round nuclei, finely dispersed chromatin, inconspicuous nucleoli, and eosinophilic cytoplasm, but their clonal relationship with the neoplastic cells has not been proven.

aCML has clinical, laboratory, and morphologic features similar to those of CML, but it differs from CML in the absence of the Philadelphia chromosome, BCR-ABL1 fusion, and the presence of dysplastic changes in one or more myeloid cell lines (Table 6.4.2) (2,4,9,10,22). aCML

can be distinguished from CMML by lower percentage of monocyte count (<10%) and higher percentage of immature myeloid cells (>10%) in the peripheral blood (Fig. 6.4.5). The blast count is usually <5% in the peripheral blood and always <20% in the bone marrow. Because of marked myelopoiesis, the M/E ratio in the bone marrow is frequently >10:1.

As the name indicates, JMML is seen in children <14 years, and 75% of patients are <3 years (2,5,9,10,23–26). It has the lowest incidence in the MDS/MPN group (1.3 per million children per year) (2), but it is most common in pediatric patients with myeloproliferative syndrome (27). Despite the age difference, JMML is similar to CMML, and some authors consider these two diseases to be synonymous.

The major laboratory features include peripheral leukocytosis (>10,000/mL), and monocytosis is present in the peripheral blood (>1,000/mL) and bone marrow (5% to 10%) (Table 6.4.3). Immature myeloid cells are also present in the peripheral blood. As in other MDS/MPN entities, the Philadelphia chromosome and BCR-ABL1 fusion are absent. Unlike CMML, myelodysplastic changes usually are not prominent, and the total leukocyte count is usually higher (2,10,27). Blasts, including promonocytes, are <20% in the peripheral blood and bone marrow, distinguishing JMML from acute myelomonocytic leukemia. However, myelomonocytic cells may infiltrate the skin, lung, liver, and spleen, mimicking acute leukemia. In nearly 70% of patients with JMML, the hemoglobin F level is >10% and the hemoglobin A₂ level is low. Experimentally, the JMML cells are able to form spontaneous granulocyte–macrophage colonies in vitro and they have marked hypersensitivity to granulocyte–macrophage colony-stimulating factor (GM-CSF) stimulation in vitro (2,23). The differences between CMML, aCML, and JMML are summarized in Table 6.4.4.

MDS/MPN unclassifiable is defined in cases that show overlapped features of both MDS and MPN and yet do not meet the criteria for CMML, aCML, and JMML (6). Cases with a preceding history of an underlying MPN or MDS, or

TABLE 6.4.2

Salient Features of Laboratory Diagnosis of aCML

1. Peripheral leukocytosis (WBC > 13,000/ μ L) with >10% immature myeloid cells
2. Prominent dysplasia in ≥ 1 myeloid lineages demonstrated in blood and/or bone marrow
3. Hypercellular bone marrow with granulocytic proliferation
4. <20% blasts in blood and bone marrow
5. No or minimal absolute monocytosis (<10% in blood)
6. Minimal absolute basophilia (<2% in blood)
7. No Philadelphia chromosome or BCR-ABL1 fusion gene
5. No rearrangement of PDGFRA or PDGFRB

TABLE 6.4.3

Salient Features for Laboratory Diagnosis of JMML

- 1. Peripheral monocytosis (>1,000/ μ L)
- 2. <20% blasts (monoblasts and promonocytes) in blood and bone marrow
- 3. No Philadelphia chromosome or BCR-ABL1 fusion gene
- 4. Two or more of the following abnormalities should be demonstrated:
 - a. Hemoglobin F increased for age
 - b. Immature granulocytes in the peripheral blood (<10%)
 - c. Leukocyte count >10,000/ μ L
 - d. Clonal chromosomal abnormality
 - e. GM-CSF hypersensitivity of myeloid progenitors in vitro

with a history of recent cytotoxic or growth factor therapy should be excluded for this diagnosis. These cases also should not have BCR-ABL1 fusion gene or rearrangement of PDGFRA, PDGFRB, or FGFR1.

RARS-T shows features of RARS, but in addition, the platelet count is $\geq 450,000/\text{mL}$. In the bone marrow, there is proliferation of large atypical megakaryocytes similar to those seen in BCR-ABL1-negative MPN. RARS-T is considered a provisional entity, because “it is not clear whether it is a distinct entity, one end of the spectrum of RARS, a progression of RARS due to an additional acquired genetic abnormality, or less likely, the occurrence of two rare diseases in the same patient” (6).

Immunophenotype and Cytochemistry

In one flow cytometric study of CMML, it was found that the monocytes showed decreased expression of monocyte-associated antigens CD13, CD15, CD36, and HLA-DR and aberrant expression of nonmyelomonocytic antigens CD2 and CD56 (28). Aberrant expression of two or more antigens may help distinguish CMML from reactive monocytosis. CMML cases also showed a significantly higher percentage (>20%) of CD14 (moderate) positive bone marrow monocytes as compared with cases of reactive monocytosis (28). CD14 (moderate) monocytes are CD45 dim, so they represent immature monocytes, whereas mature monocytes are CD14 (strong). The combination of two or more immunophenotypic aberrations and $\geq 20\%$ CD14 (moderate) bone marrow monocytes was 67% sensitive and 100% specific for the diagnosis of CMML (28).

To distinguish CMML from acute myelomonocytic leukemia, the blasts count is the major criterion and 20% of blasts is the cutoff point. Yang et al. (29) suggested using a panel of markers to separate different stages of monocytes. The entire monocyte population can be isolated by dual bright CD33 and CD64 staining. CD64 is positive for all mature and immature monocytes. CD14 has two epitopes: My4 and Mo2. My4 is present in mature monocytes and promonocytes. Mo2 is only expressed by mature monocytes. Therefore, dual staining with these markers can separate mature monocytes from promonocytes and monoblasts. The plasmacytoid monocytes (plasmacytoid dendritic cells) in the lymph nodes of CMML cases also show a specific immunophenotype. These cells are positive for CD4, CD14, CD43, CD56, CD68, and CD123 (15,18–21).

There have been no reports of specific immunophenotypes for aCML and JMML. However, the immunophenotype as described in CMML is also applicable to the monocytes in JMML. In aCML, the myeloid cells can be identified by CD13, CD33, and myeloperoxidase and myeloblasts can

TABLE 6.4.4

Comparison of CMML, aCML, and JMML

Feature	CMML	aCML	JMML
Philadelphia chromosome	Negative	Negative	Negative
BCR-ABL1	Negative	Negative	Negative
Peripheral monocytes	>1,000/ mL	$\geq 3\%$ to <10%	>1,000/ mL
Marrow monocytes	$\geq 10\%$	$\leq 3\%$	5%–10%
Peripheral immature myeloid cells	$\leq 10\%$	10% to 20%	$\leq 10\%$
Peripheral basophils	<2%	<2%	<2%
Granulocytic dysplasia	Marked	Very marked	Minimal
Marrow erythroid precursors	>15%	Low percentage	Low percentage

CMML, chronic myelomonocytic leukemia; aCML, atypical chronic myeloid leukemia; JMML, juvenile myelomonocytic leukemia; BCR-ABL, breakpoint cluster region/Ableson.

be estimated by CD34 and CD117. Monoblasts, however, are negative or weakly positive for CD34 and CD117 in many cases.

Immunohistochemical stains, such as CD33, CD68 (PG-M1) (Fig. 6.4.6), CD163 (Fig. 6.4.7), and myeloperoxidase, are helpful in demonstrating the myelomonocytic components. However, cytochemical stains with myeloperoxidase, lysozyme, a-naphthyl acetate esterase and a-naphthyl butyrate esterase (Fig. 6.4.8) on peripheral blood or bone marrow smears are most useful in distinguishing myelocytes from monocytes. Leukocyte alkaline phosphatase scores are decreased in 50% of JMML cases, but are variable in aCML cases (2).

Comparison between Flow Cytometry and Immunohistochemistry

Morphologic identification of various abnormal monocytes is difficult and subject to significant variations between observers. Therefore, immunophenotype and cytochemical stains are of utmost importance to facilitate an accurate count of monocytes. With a large panel of myelomonocytic markers, flow cytometry can isolate monocytes from myelocytes and identify different developmental stages, so that it is most helpful in substantiating the diagnosis of CMML and JMML. Cytochemical staining with esterases has the advantage of correlating the markers with morphology, but some immature monocytic cells may not show nonspecific esterase staining thus underestimating the monocyte count.

Immunohistochemical staining is not as helpful as cytochemical stains in terms of morphologic correlation. However, CD68 (KP1) and the new marker CD163 can help to demonstrate monocytosis in the bone marrow (15). CD34 may be useful in distinguish CMML1 from CMML2. CD42b is used to identify dwarf megakaryocytes, which may not be recognizable in H&E sections. Finally, CD123 may help to detect the plasmacytoid dendritic cell nodules, which is only seen in CMML and not other MPN cases (15).

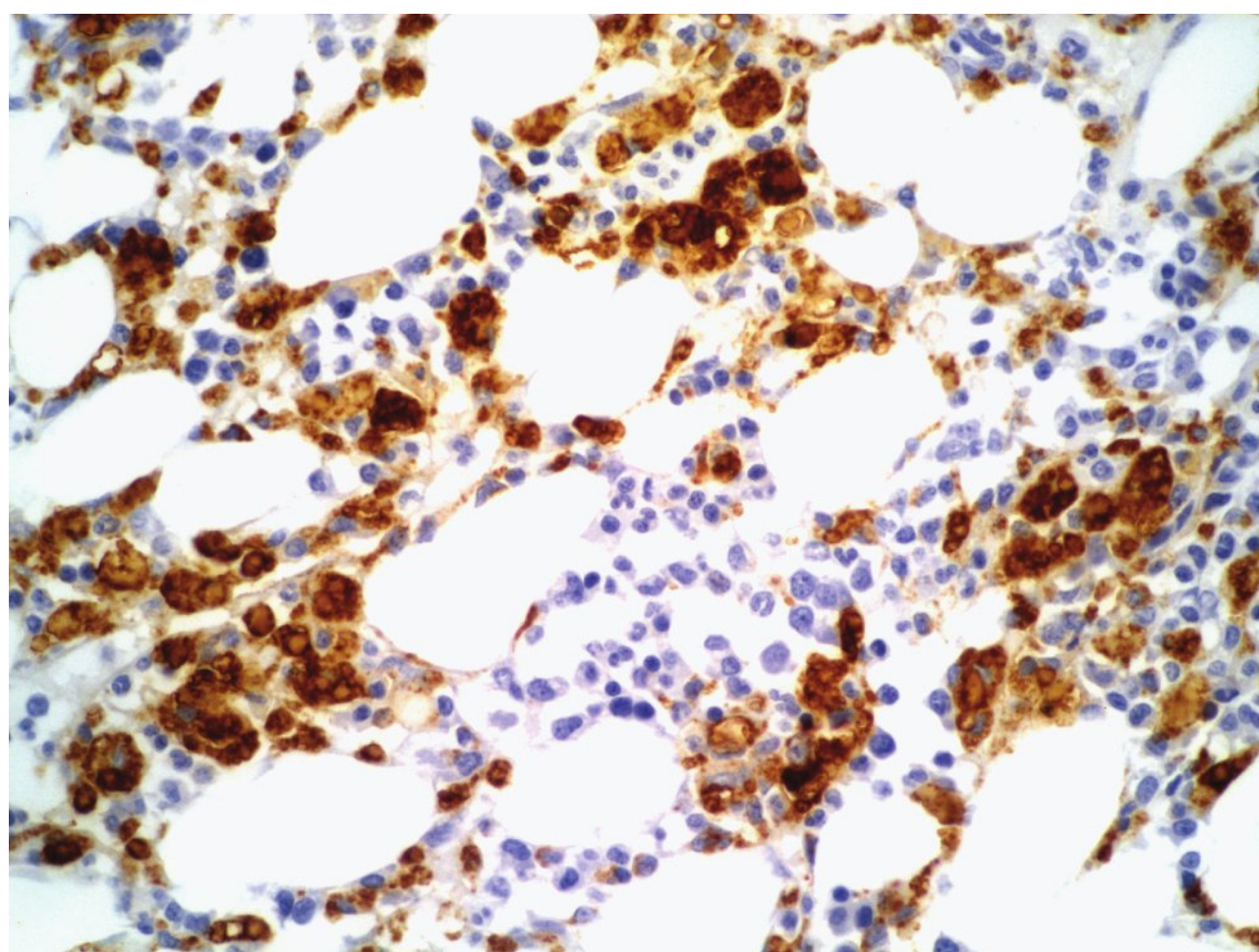


FIGURE 6.4.6 Bone marrow core biopsy from a case of CMML shows positive CD68 PG-M1 staining in many monocytes. Immunoperoxidase, 40× magnification.

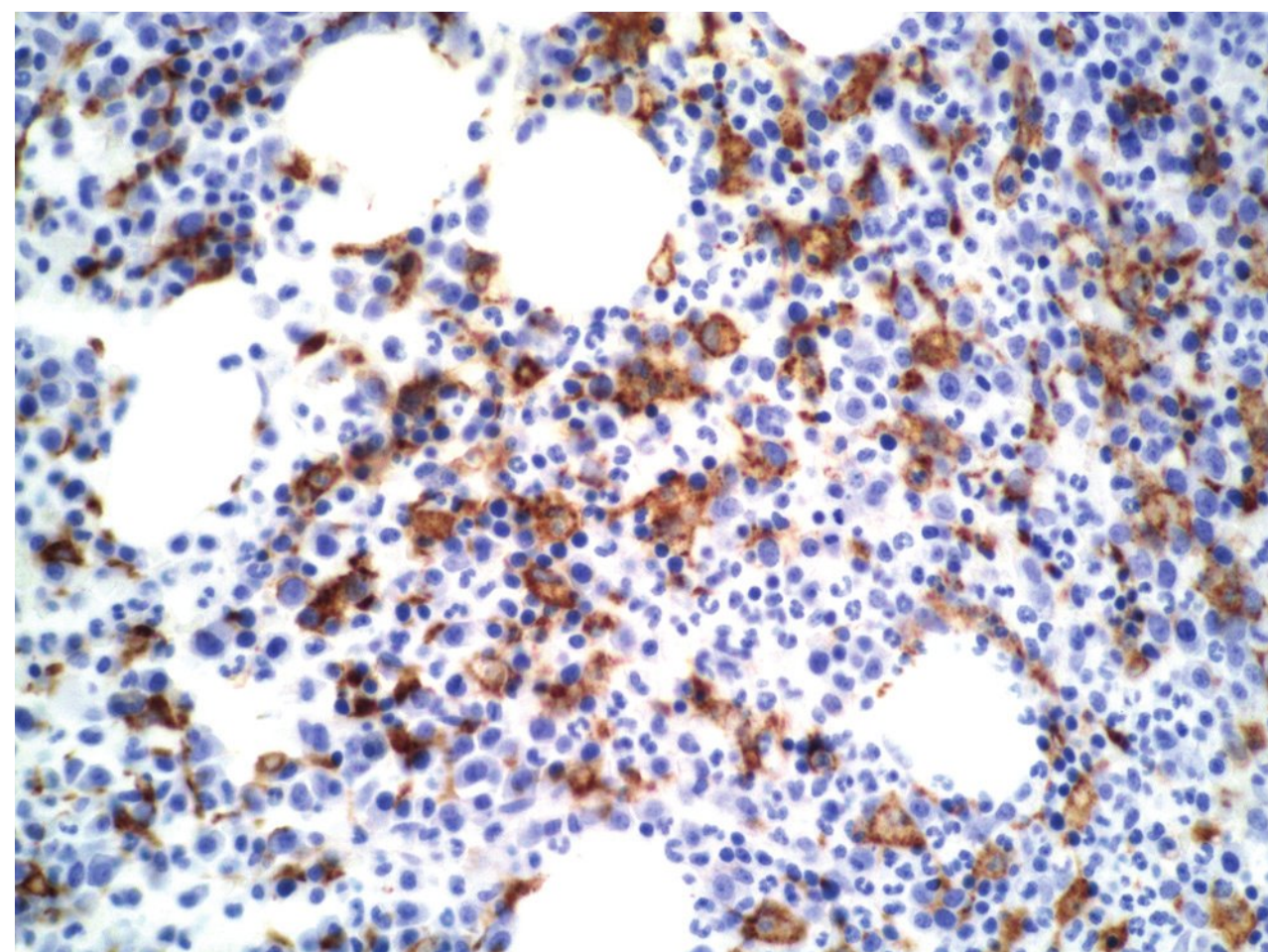


FIGURE 6.4.7 Bone marrow biopsy from a case of myelomonocytic leukemia shows positive CD163 staining in many monocytes. Immunoperoxidase, 40× magnification.

Molecular Genetics

Approximately 20% to 30% of CMML cases have aberrant karyotypes, including trisomy 8, del(20q), monosomy 7, and del(11q), but these abnormalities can also be seen in other myeloproliferative or myelodysplastic disorders (2,10). However, a subset of CMML, CMML with blood and marrow eosinophilia, is associated with t(5:12)(q33;p13), which is specific (16,17). Molecular characterization has revealed that this translocation involves the TEL gene on chromosome 12 and the platelet-derived growth factor receptor (PDGFR) gene on chromosome 5 (30). The resultant TEL-PDGFR fusion transcript may play an important role in the proliferative process, probably through the deregulation of an oncogene derived from rat sarcoma virus (RAS) (10). Point mutation of RAS genes has been found in as many as 40% of CMML patients (2). Translocations of the PDGFR

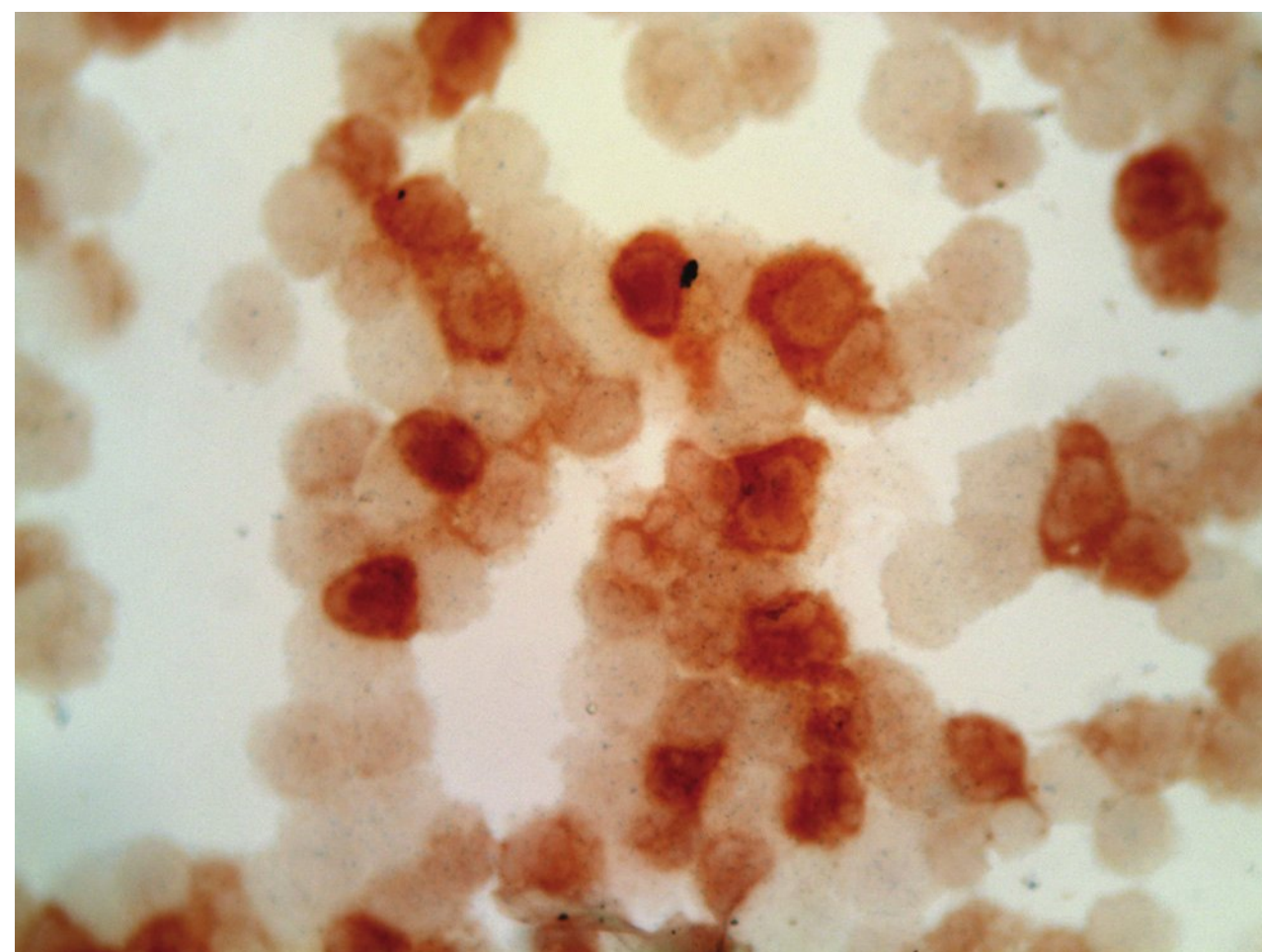


FIGURE 6.4.8 Cytospin smear of bone marrow aspirate from a patient of JMML shows positive staining of a-naphthyl butyrate esterase in many monocytes. Cytochemical stain, 60× magnification.

gene to other partner genes have also been reported (31). A recent report demonstrated recurrent somatic activating mutation in the Janus kinase 2 (JAK2) tyrosine kinase in 9 of 116 CMML/aCML cases (32). CCAAT/enhancer binding protein a (CEBPa) gene mutation is involved in CMML cases transforming into acute myeloid leukemia (33).

A summary of molecular findings in CMML include NRAS or KRAS mutations in about one third of patients, TET2 mutations in up to 40% of patients, RUNX mutations in about 40% of patients, ASXL1 mutations in patients with high leukocyte counts, CML mutations in about 10% of patients, and JAK2 (V617F) in occasional patients (34). In terms of micro RNA pattern, down-regulation of miR-10a is found in CMML (35).

As many as 80% of patients with aCML have cytogenetic abnormalities, including +8, +13, +14, del(20q), i(17q), and del(12q), but none of them are specific (2,10). As mentioned above, JAK2 mutation has also been found in aCML patients (32). t(9;15;12) translocation involving the ETV6 gene at 12q13 and the JAK2 gene at 9q24 has been reported in a case of aCML in transformation (36). The major molecular findings in aCML include NRAS or KRAS mutations and TET2 mutations in about one third of patients and CBL mutations in about 10% of patients (34). The findings in microRNA pattern include down-regulation of miR-10a and overexpression of miR-424 (35).

Cytogenetic abnormalities occur in 30% to 40% of JMML patients, but none of them are specific (2). Monosomy 7 is frequently associated with JMML, but the relationship of JMML and childhood monosomy 7 syndrome is uncertain (23). The most important genetic finding in JMML is point mutation of the RAS gene, which is present in 20% to 30% of patients (2,10). These point mutations may induce the increase of intracellular levels of RAS–guanosine 5′-triphosphate (GTP), which may alter the RAS signaling pathway. In JMML patients with neurofibromatosis type 1 (NF1), loss of the normal NF1 allele is a common finding in the leukemic cells. The normal NF1 protein, neurofibromin, down-regulates RAS-GTP. Inactivation of NF1 may deregulate the RAS pathway. In addition, somatic mutations of PTPN11 have been reported in 35% of JMML patients (37). It is believed that NRAS, KRAS, CBL, NF1, and PTPN11 play an important role in the pathogenesis of JMML (38).

The current case showed both myeloproliferative and myelodysplastic features with peripheral and bone marrow monocytosis, so it was consistent with CMML morphologically. The positive α-naphthyl butyrate esterase staining in the bone marrow aspirate smear and positive CD68 (PG-M1) staining in the core biopsy further confirmed this diagnosis. In addition, the patient also had eosinophilia in the peripheral blood and bone marrow. CMML with eosinophilia is associated with t(5;12), but it occurs in only 1% to 2% of such cases (2).

Clinical Manifestations

Patients with CMML may have fatigue, weight loss, fever, and night sweats (2,3). In addition, they may have recurrent infections due to dysfunctional leukocytes and hemorrhages due to thrombocytopenia. Many patients have splenomegaly and/or hepatomegaly (2,3,10). The survival time of these

patients may vary from 1 month to >100 months with a median survival of 20 to 40 months in most studies (2,3). The most important prognostic predictor is the number of blasts.

There are no specific clinical symptoms reported in aCML cases. Those presenting symptoms may be related to anemia, thrombocytopenia, or splenomegaly. The median survival times reported are <20 months (2,4). About 25% to 40% of aCML cases evolve into acute leukemia.

Clinical symptoms in JMML patients include malaise, pallor, fever, or other evidence of infection (2,5,10). Other symptoms may be related to bleeding or pulmonary involvement. A maculopapular skin rash may be present in ≤50% of patients. Splenomegaly is virtually always present. Hepatomegaly and lymphadenopathy are found in more than one half of patients (10). Patients with JMML and those with childhood monosomy 7 syndrome have similar clinical symptoms. JMML without monosomy 7 syndrome tend to have higher hemoglobin F levels, more prominent lymphadenopathy, and more severe skin rashes than do patients with monosomy 7 syndrome, whereas the latter have a tendency to develop leukopenia and bacterial infections (23). The median survival times vary from 5 months to >4 years, depending on the treatment the patients received (2). If untreated, 30% of patients die within 1 year of diagnosis.

REFERENCES

1. Neuwirtova R, Mocikova K, Musilova J, et al. Mixed myelodysplastic and myeloproliferative syndromes. *Leuk Res*. 1996;20:717–726.
2. Vardiman JW. Myelodysplastic/myeloproliferative diseases. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:47–59.
3. Orazi A, Bennett JM, Germing U, et al. Chronic myelomonocytic leukaemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:76–79.
4. Vardiman JW, Bennett JM, Bain BJ, et al. Atypical chronic myeloid leukaemia, BCR-ABL1 negative. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:80–81.
5. Baumann I, Bennett JM, Niemeyer CM, et al. Juvenile myelomonocytic leukaemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:82–84.
6. Vardiman JW, Bennett JM, Bain BJ, et al. Myelodysplastic/myeloproliferative neoplasm, unclassifiable. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:85–86.
7. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
8. Bennett JM, Catovsky D, Daniel MT, et al. The chronic myeloid leukaemias: guidelines for distinguishing chronic granulocytic, atypical chronic myeloid, and

- chronic myelomonocytic leukaemia. Proposals by the French-American-British Cooperative Leukaemia Group. *Br J Haematol*. 1994;87:746–754.
9. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292–2302.
 10. Anastasi J, Vardiman JW. Chronic myelogenous leukemia and the chronic myeloproliferative diseases. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1745–1790.
 11. Orazi A, Germing U. The myelodysplastic/myeloproliferative neoplasms: myeloproliferative diseases with dysplastic features. *Leukemia*. 2008;22:1308–1319.
 12. Foucar K. Myelodysplastic/myeloproliferative neoplasms. *Am J Clin Pathol*. 2009;132:281–289.
 13. Xubo G, Xingguo L, Xianguo W, et al. The role of peripheral blood, bone marrow aspirate and especially bone marrow trephine biopsy in distinguishing atypical chronic myeloid leukemia from chronic granulocytic leukemia and chronic myelomonocytic leukemia. *Eur J Haematol*. 2009;83:292–301.
 14. Ngo NT, Lampert IA, Naresh KN. Bone marrow trephine morphology and immunohistochemical findings in chronic myelomonocytic leukaemia. *Br J Haematol*. 2008;141:771–781.
 15. Orazi A, Chiu R, O'Malley DP, et al. Chronic myelomonocytic leukemia: the role of bone marrow biopsy immunohistology. *Mod Pathol*. 2006;19:1536–1545.
 16. Baranger L, Szapiro N, Gardais J, et al. Translocation t(5;12)(q31-q33;p12-p13): a non-random translocation associated with a myeloid disorder with eosinophilia. *Br J Haematol*. 1994;88:343–347.
 17. Hyde J, Sun T. Chronic myelomonocytic leukemia with abnormal bone marrow eosinophils. *Arch Pathol Lab Med*. 2003;127:1214–1216.
 18. Facchetti F, De Wolf-Peeters C, Kennes C, et al. Leukemia-associated lymph node infiltrates of plasmacytoid monocytes (so-called plasmacytoid T-cells). Evidence for two distinct histological and immunophenotypical patterns. *Am J Surg Pathol*. 1990;14:101–112.
 19. Harris NL, Demirjian Z. Plasmacytoid T-zone cell proliferation in a patient with chronic myelomonocytic leukemia. Histologic and immunohistologic characterization. *Am J Surg Pathol*. 1991;15:87–95.
 20. Baddoura FK, Hanson C, Chan WC. Plasmacytoid monocyte proliferation associated with myeloproliferative disorders. *Cancer*. 1992;69:1457–1467.
 21. Horny HP, Kaiserling E, Handgretinger R, et al. Evidence for a lymphotropic nature of circulating plasmacytoid monocytes: finding from a case of CD56+ chronic myelomonocytic leukemia. *Eur J Haematol*. 1995;54:209–216.
 22. Hernandez JM, del Canizo MC, Cunco A, et al. Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. *Ann Oncol*. 2000;11:441–444.
 23. Arico M, Biondi A, Pui CH. Juvenile myelomonocytic leukemia. *Blood*. 1997;90:479–488.
 24. Niemeyer CM, Arico M, Basso G, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. *Blood*. 1997;89:3534–3543.
 25. Niemeyer CM, Fenu S, Hasle H, et al. Response: differentiating juvenile myelomonocytic leukemia from infectious disease. *Blood*. 1998;91:365–366.
 26. Chang YH, Jou ST, Lin DT, et al. Differentiating juvenile myelomonocytic leukemia from chronic myeloid leukemia in childhood. *J Pediatr Hematol Oncol*. 2004;26:236–242.
 27. Gassas A, Doyle JJ, Weitzman S, et al. A basic classification and a comprehensive examination of pediatric myeloproliferative syndromes. *J Pediatr Hematol Oncol*. 2005;27:192–196.
 28. Xu Y, McKenna RW, Karandikar NJ, et al. Flow cytometric analysis of monocytes as a tool for distinguishing chronic myelomonocytic leukemia from reactive monocytosis. *Am J Clin Pathol*. 2005;124:799–806.
 29. Yang DT, Greenwood JH, Hartung L, et al. Flow cytometric analysis of different CD14 epitopes can help identify immature monocytic populations. *Am J Clin Pathol*. 2005;124:930–936.
 30. Golub RR, Barker GF, Lovett M, et al. Fusion of PDGF receptor beta to a novel ets-like gene, tel, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell*. 1994;77:307–316.
 31. Wlodarska I, Mecucci C, Marynen P, et al. TEL gene is involved in myelodysplastic syndromes with either the typical t(5;12)(q33;p13) translocation or its variant t(10;12)(q24;p13). *Blood*. 1995;85:2848–2852.
 32. Levine RL, Loriaux M, Huntly BJP, et al. The JAK2V617F activating mutation occurs in chronic myelomonocytic leukemia and acute myeloid leukemia, but not in acute lymphoblastic leukemia or chronic lymphocytic leukemia. *Blood*. 2005;106:3377–3379.
 33. Shih LY, Huang CF, Lin TL, et al. Heterogeneous patterns of CEBPa mutation status in the progression of myelodysplastic syndrome and chronic myelomonocytic leukemia to acute myelogenous leukemia. *Clin Cancer Res*. 2005;11:1821–1826.
 34. Reiter A, Invernizzi R, Cross NCP, et al. Molecular basis of myelodysplastic/myeloproliferative neoplasms. *Haematologica*. 2009;94:1634–1638.
 35. Hussein K, Busche G, Muth M, et al. Expression of myelopoiesis-associated microRNA in bone marrow cells of atypical chronic myeloid leukaemia and chronic myelomonocytic leukaemia. *Ann Hematol*. 2010;90:307–313.
 36. Peeters P, Raynaud SD, Cools J, et al. Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. *Blood*. 1997;90:2535–2540.
 37. Kratz CP, Niemeyer CM, Castleberry RP, et al. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. *Blood*. 2005;106:2183–2185.
 38. Sugimoto Y, Muramatsu H, Makishima H, et al. Spectrum of molecular defects in juvenile myelomonocytic leukaemia includes ASXL1 mutations. *Br J Haematol*. 2010;150:83–87.

CASE 5

Acute Myeloid Leukemia with t(8;21)(q22;q22)

CASE HISTORY

A 19-year-old man presented with a 4-week history of sore throat, fever to 103°F, lethargy, chest congestion, and flu-like symptoms. The day before admission, he noticed the onset of widespread petechiae. He had been seen in the clinic 1 week before and was given erythromycin for a presumed upper respiratory infection. Physical examination showed pallor, petechiae, diffuse lymphadenopathy with 4+ tonsillar hypertrophy, and mild hepatosplenomegaly. Hematology workup revealed a total leukocyte count of 128,000/mL with 97% blasts, 2% segmented neutrophils, and 1% lymphocytes. The hematocrit was 36% and platelet count, 23,000/mL. Lactate dehydrogenase was 555 U/L. A bone marrow biopsy and aspirate were performed.

FLOW CYTOMETRY FINDINGS

Blood: Myeloid markers: myeloperoxidase 87%, CD13-CD33 97%. Monocyte marker: CD14 0%. Activation antigen: HLA-DR 0%. T-cell marker: CD7 0%. Stem cell marker: CD34 20%.

Bone marrow: Myeloid markers: myeloperoxidase 100%, CD13-CD33 93%. Monocyte marker: CD14 59%. Activation antigen: HLA-DR 86%. T-cell marker: CD7 0%. B-cell marker: CD19 0%. Stem cell marker: CD34 60% (Fig. 6.5.1).

CYTOCHEMICAL FINDINGS

The blasts were positive for myeloperoxidase and chloroacetate esterase, but negative for α-naphthyl butyrate esterase.

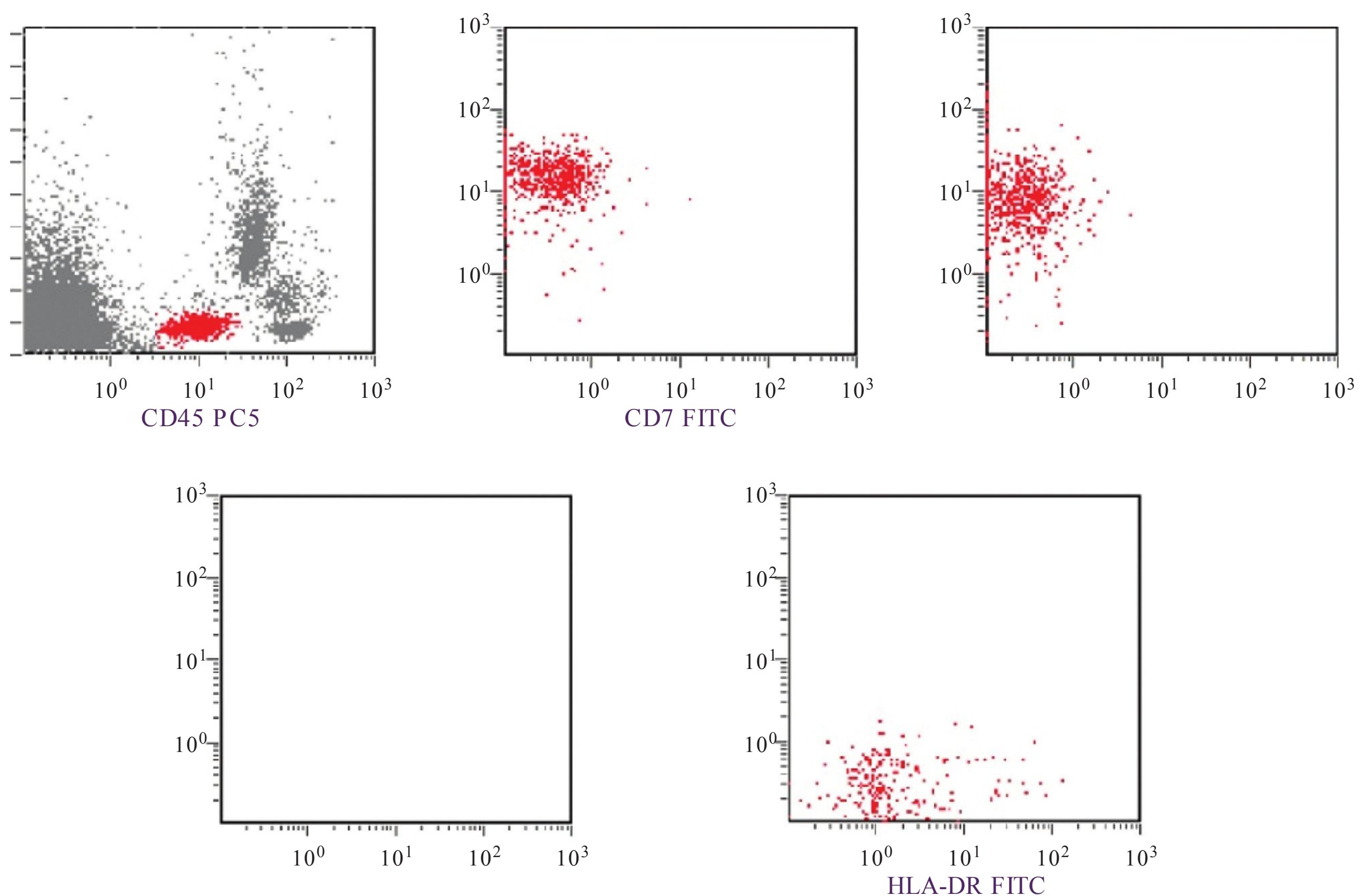


FIGURE 6.5.1 Flow cytometric analysis of bone marrow shows positive CD13, CD33, CD34, and HLA-DR, but negative CD7 and CD117. Note that a large population of mature myeloid cells is present above the gated acute myeloid leukemia (AML) population. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; RD1, rhodamine; FITC, fluorescein isothiocyanate.

MOLECULAR GENETIC STUDIES

Fluorescence in situ hybridization (FISH) for t(15;17) was negative. Karyotyping showed t(8;21)(q22;q22) (Fig. 6.5.2).

DISCUSSION

In the current case, the peripheral blood showed many blasts containing multiple Auer rods, and the bone marrow revealed 92% blasts with >10% of type 3 blasts (blasts that contain >20 cytoplasmic granules) (Figs. 6.5.3 and 6.5.4). The presence of hypergranular myeloid cells with multiple Auer rods and an immunophenotype of negative HLA-DR misled us to consider acute promyelocytic leukemia and triggered the order of FISH for t(15;17), the result of which was negative. The karyotype of t(8;21) together with the above-described morphology finally provided a definitive diagnosis of acute myeloid leukemia with maturation (AML-M2). The immunophenotyping of the bone marrow showed a normal percentage of HLA-DR, indicating that the absence of HLA-DR in the immunophenotype of the peripheral blood specimen was probably a technical error. The identification of this particular karyotype is clinically important because it confirms the diagnosis of AML even when the blast count in the bone marrow is <20% (1). It also confers a favorable prognosis. The presence of type 3 blasts

defines the leukemia as AML-M2 irrespective of whether the mature myeloid cell count is below or above 10% (2).

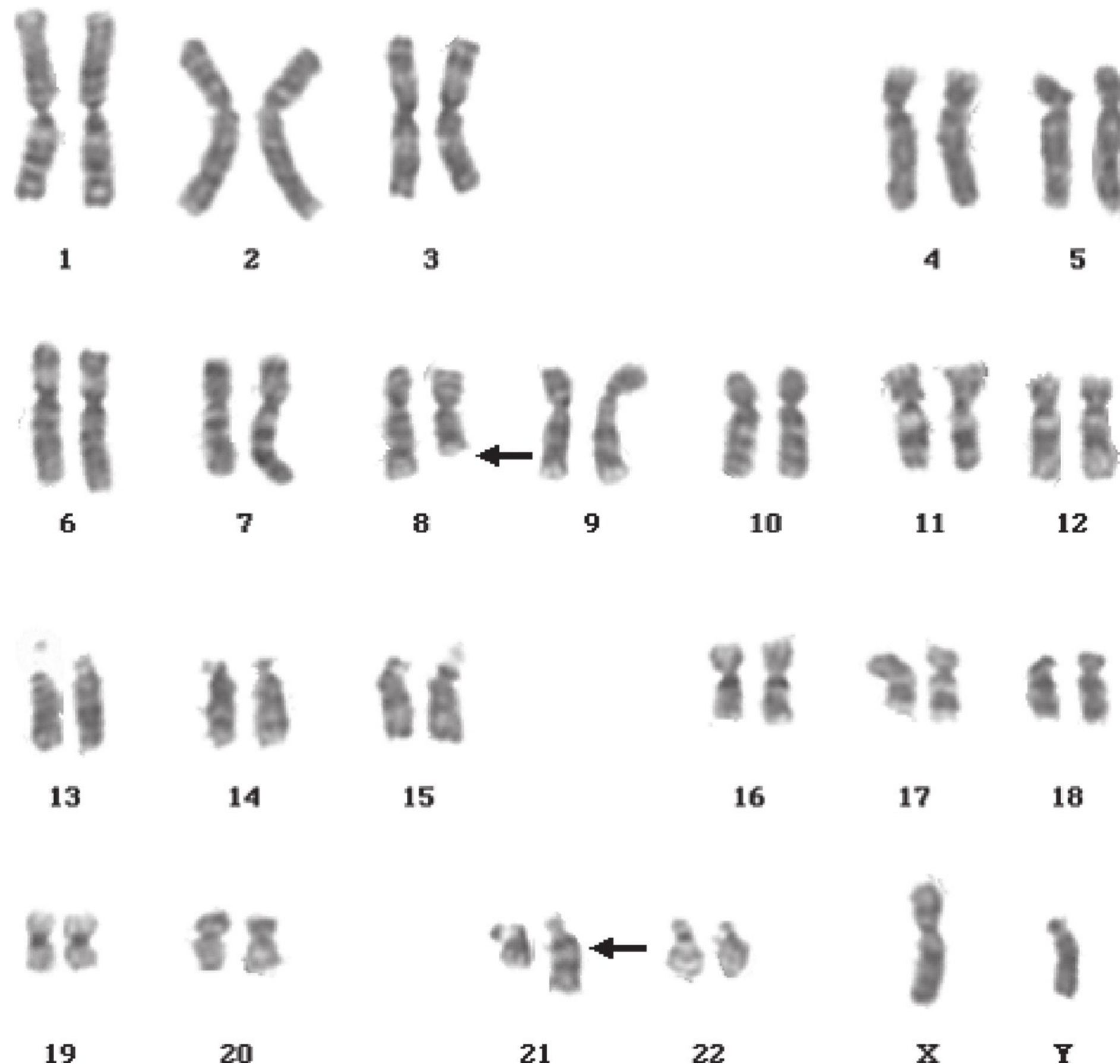
Morphology

The hematologic features in this particular genotype are characterized by abundant Auer rods (Fig. 6.5.5) or Auer rods with a single long and sharp rod with tapered ends, strong myeloperoxidase activity, salmon-colored cytoplasmic granules, and a rim of basophilic cytoplasm in maturing leukemic cells, large cytoplasmic vacuoles, and bone marrow eosinophilia (3). The eosinophils may show periodic acid-Schiff–positive granules. Nucifora et al. (4) added two more parameters: the French-American-British (FAB) M2 subtype and cells containing pink, waxy inclusions approximately 2 to 3 mm in diameter, as the seven predictive criteria for a t(8;21) or AML/ETO (eight twenty-one) translocation.

Andrieu et al. (5) developed a weighted score system including FAB-M2 subtype, Auer rods, pseudo-Chediak–Higashi anomaly (Fig. 6.5.6), marrow eosinophilia, large blasts with prominent Golgi (Fig. 6.5.7), and abnormal cytoplasmic granules. The sensitivity of this system is claimed to be 100%, but the false-positive rate is 7%.

In addition, >10% type 3 blasts may be present in AML-M2 including this special subtype (2). Auer rods can be demonstrated in mature granulocytes as well as eosinophils (1). Myelodysplastic changes, such as pseudo-Pelger–Huet

FIGURE 6.5.2 Karyotype of bone marrow reveals t(8;21)(q22;q22) (arrows). (Courtesy of Peter Papenhausen, Ph.D., LabCorp of America Cytogenetic Department, North Carolina).



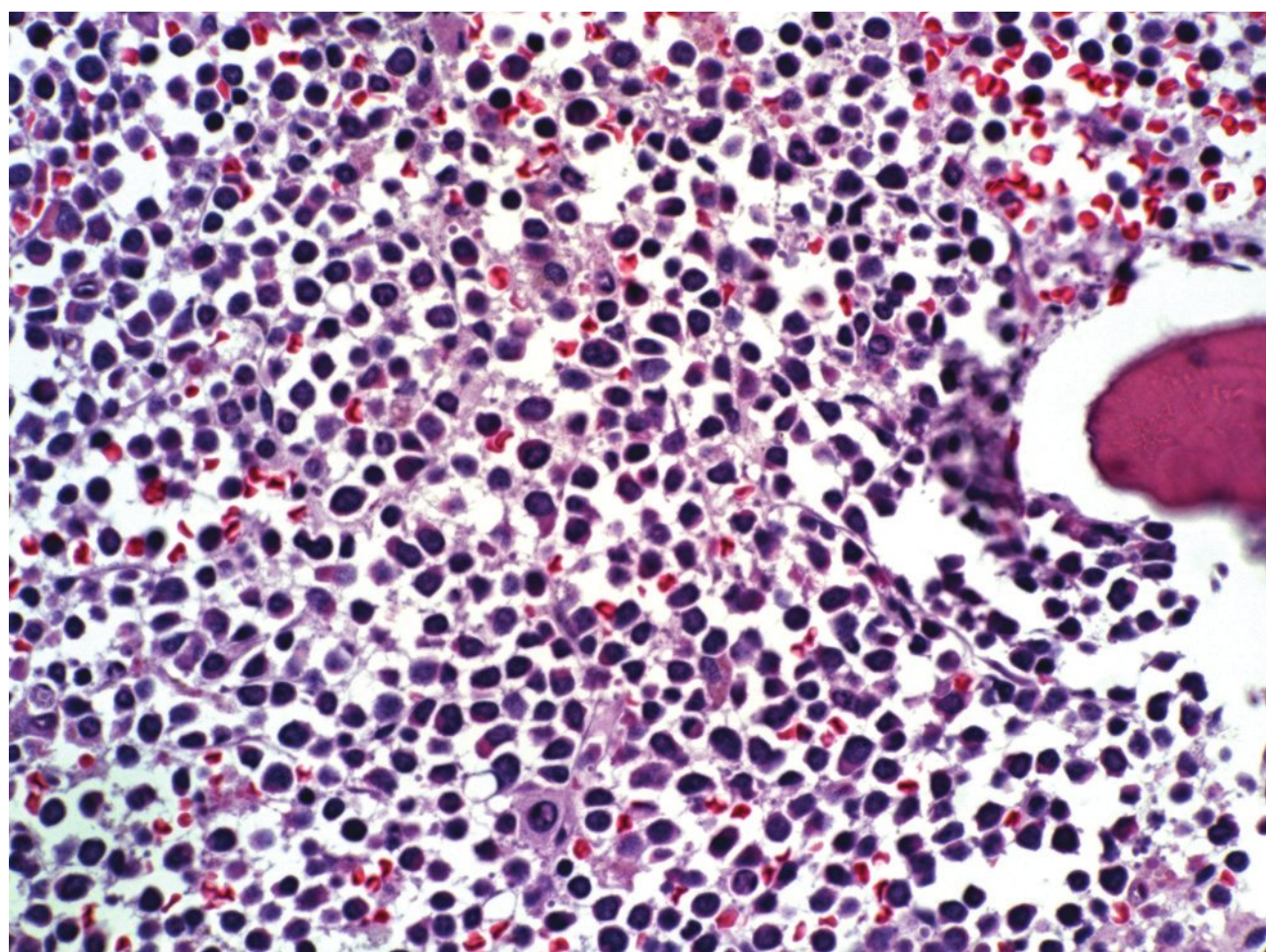


FIGURE 6.5.3 Bone marrow core biopsy shows extensive immature myeloid cell infiltration replacing normal hematopoietic cells. No normoblasts and megakaryocytes are present. Hematoxylin and eosin, 40× magnification.

cells and hypogranular neutrophils, may be demonstrated in the myeloid series. The special morphologic features are summarized in Table 6.5.1.

The cytochemical characteristics are similar to other myeloid leukemia showing the presence of positive reaction to myeloperoxidase and chloroacetate esterase, but absence of a-naphthyl butyrate esterase.

AML cases with t(8;21) have a high frequency of developing into myeloid sarcoma (6). In a study of 84 patients with t(8;21), 8 had extramedullary myeloid leukemia, mainly involving the spinal cord (7). Myeloid sarcoma may also be the initial clinical presentation. In such cases, the bone marrow may show a misleadingly low number of blast cells, but they should still be diagnosed as AML (8).

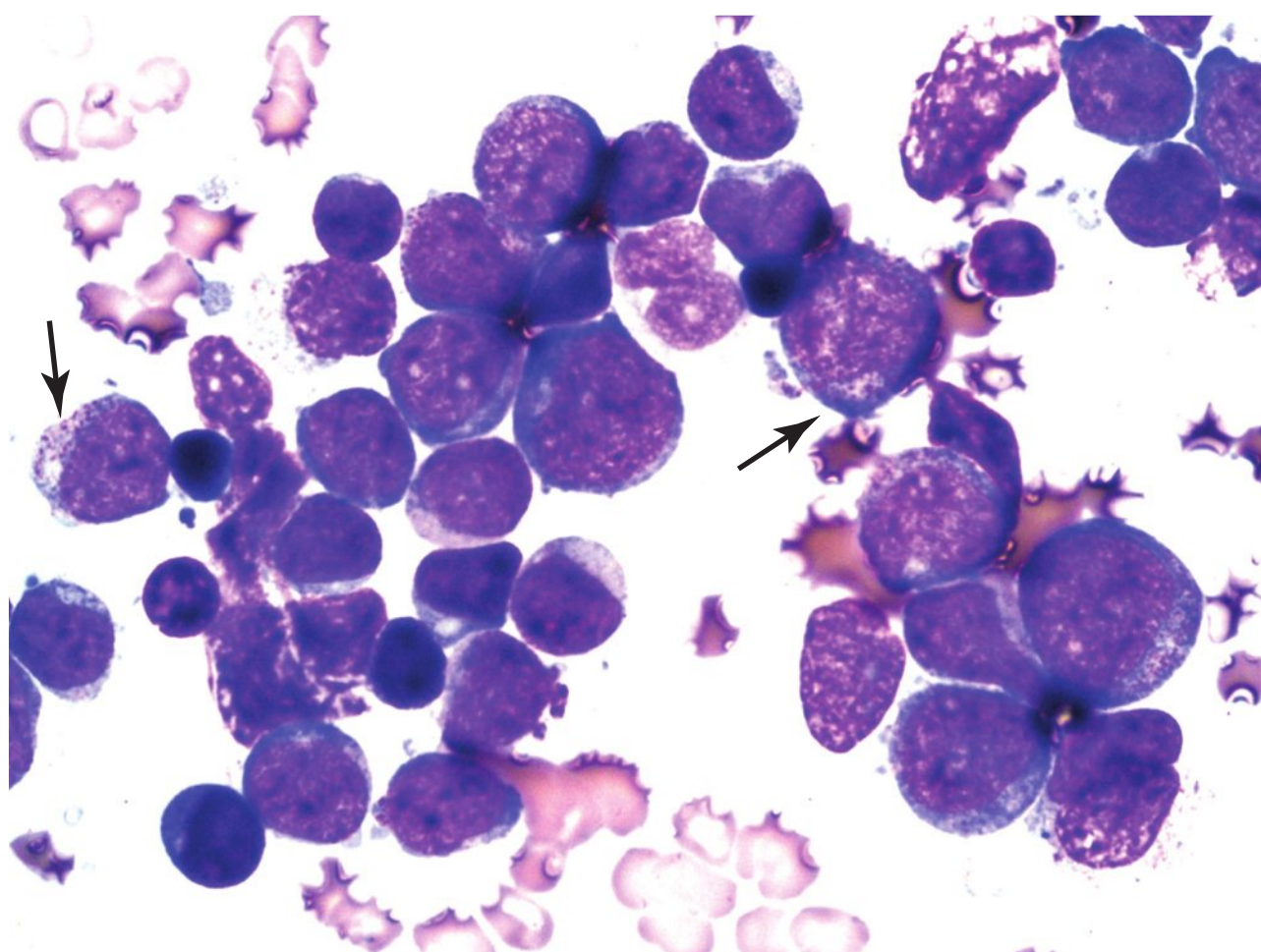


FIGURE 6.5.4 Bone marrow aspirate reveals a cluster of myeloblasts with several showing >20 cytoplasmic granules (type 3 blasts) (arrows). Wright–Giemsa, 100× magnification.

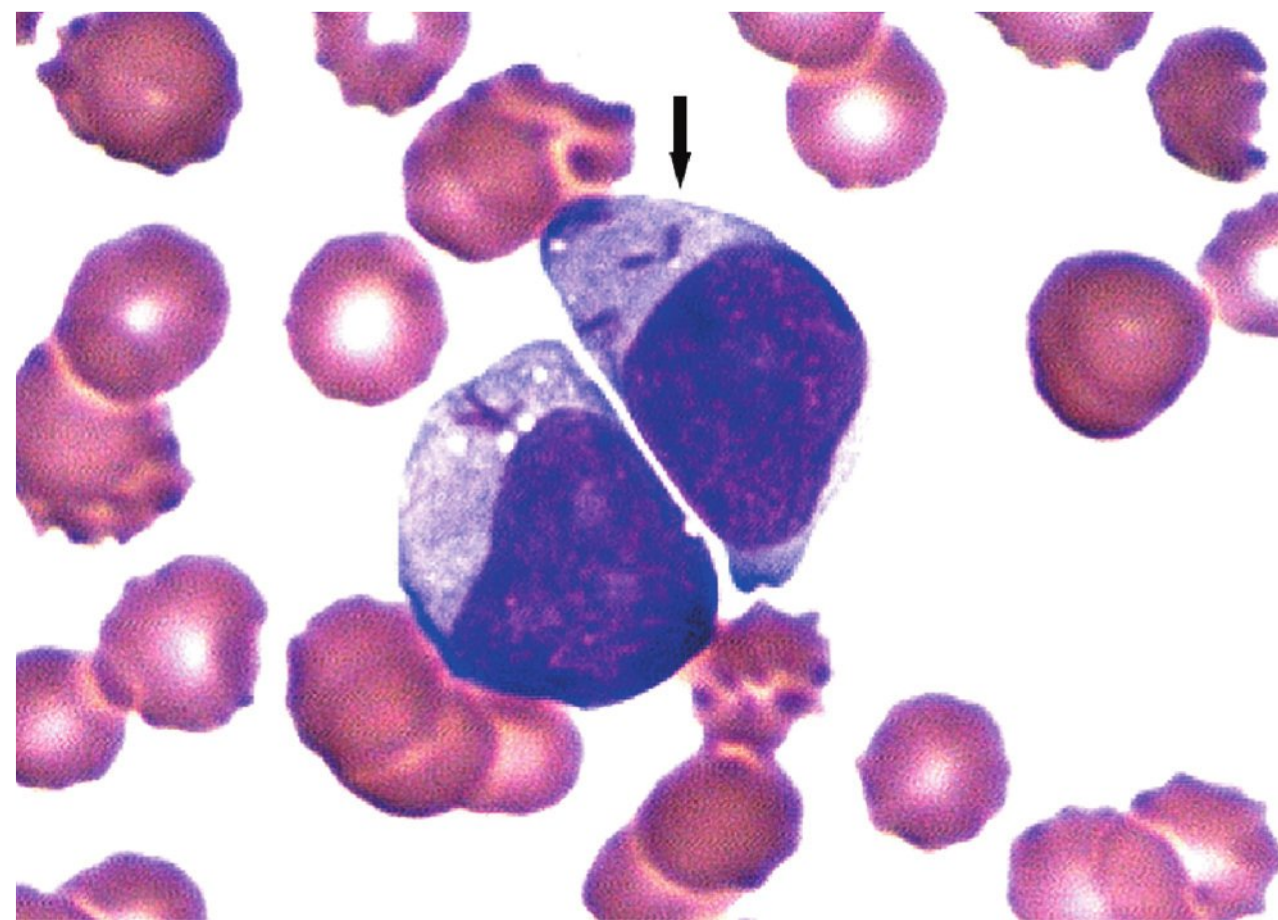


FIGURE 6.5.5 Peripheral blood smear shows two immature myeloid cells with multiple Auer rods (arrow). Wright–Giemsa, 100× magnification.

Immunophenotype

An AML case with t(8;21) expresses the same myeloid antigens (such as CD13, CD15, CD33, and myeloperoxidase) as other AML subtypes (1). However, there are also some specific markers for this special subtype. The most important one is the B-cell antigen, CD19, which is present in a subset of blasts (1,8). Another unusual marker is a natural killer cell marker, CD56, which is not as frequently demonstrated as CD19, but its presence confers an adverse prognosis (9,10). The stem cell marker CD34 is characteristically present and may help to identify its malignant nature. C-KIT gene mutation and overexpression are found in this special subtype of AML, and its protein product, CD117, is expressed on the leukemic cells as demonstrated by immunohistochemistry (11).

In a study of 93 cases of AML with t(8;21), it was shown that the cases are characterized by a significantly higher

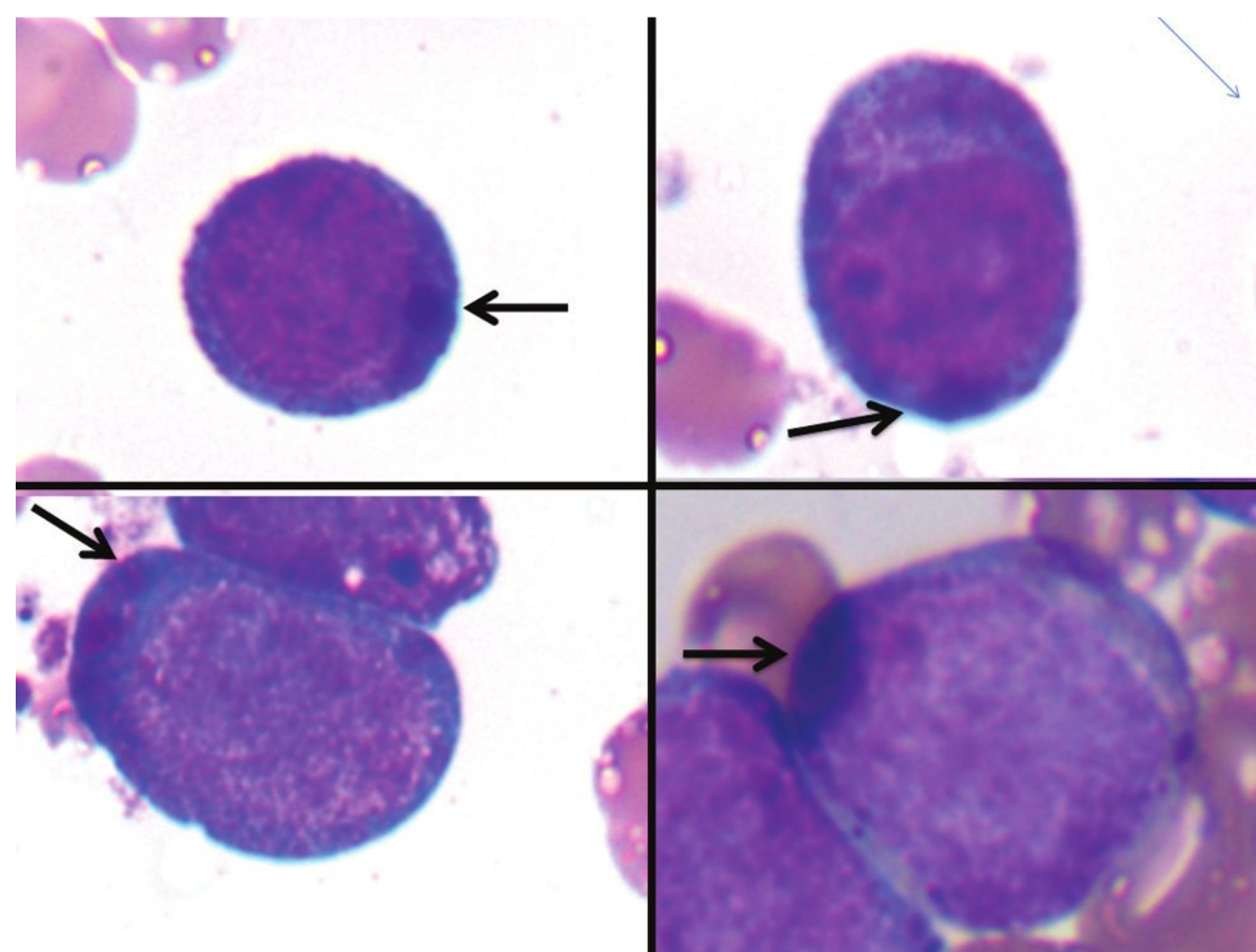


FIGURE 6.5.6 Four myeloblasts containing large cytoplasmic lysosomes (pseudo-Chediak–Higashi anomaly) (arrow) are from a bone marrow aspirate. Wright–Giemsa, 100× magnification.

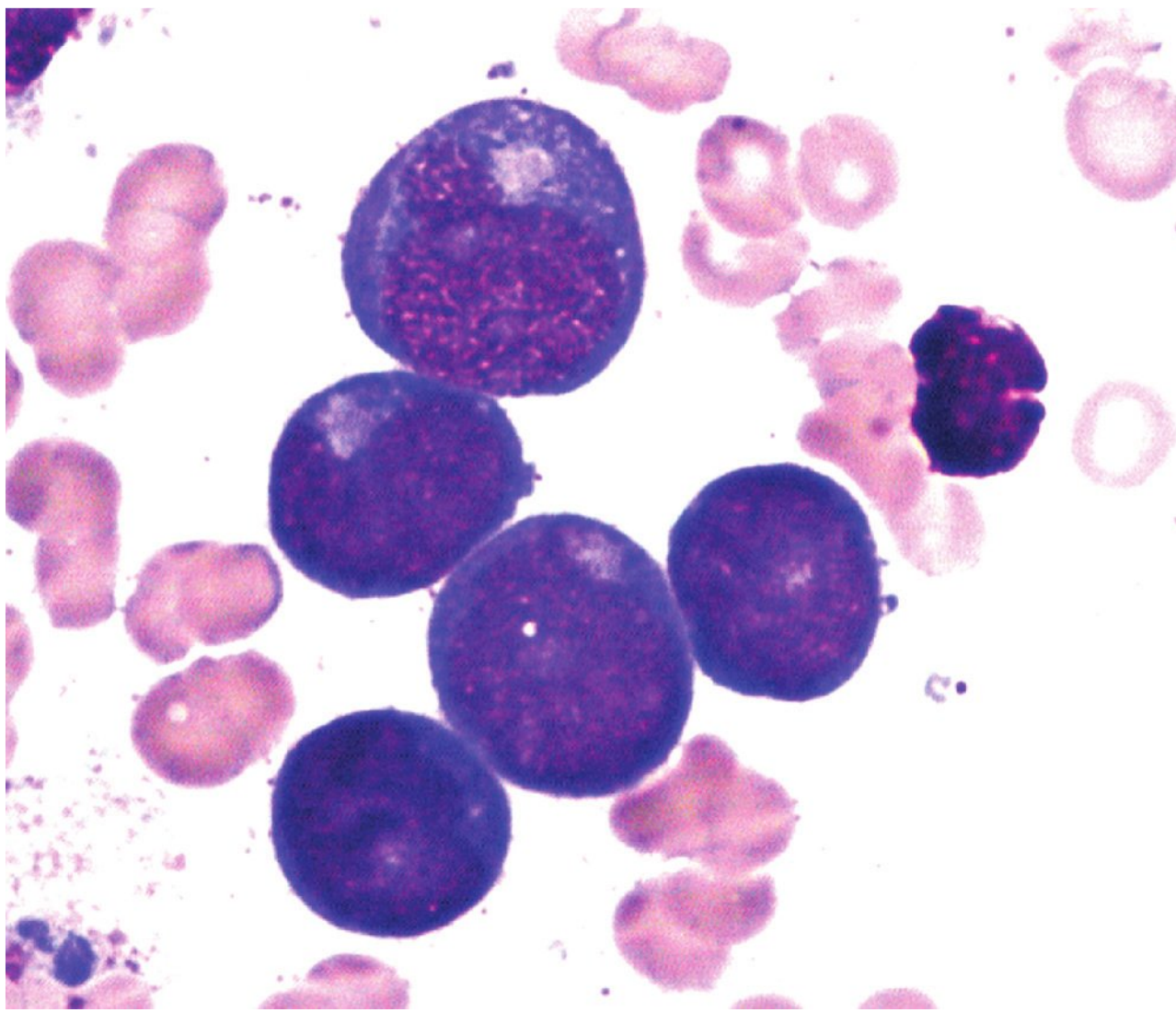


FIGURE 6.5.7 Bone marrow aspirate shows five myeloblasts with prominent Golgi. Wright–Giemsa, 200× magnification.

expression of CD19, CD34, CD56, and CD54 than are other AML subtypes with normal or other abnormal karyotypes (12). Conversely, CD45RO, CD33, CD36, CD11b, and CD14 were significantly lower in t(8;21) cases than in controls. In other studies, however, CD33 was often expressed in low intensity (5,13,14). T-cell markers, such as CD2 and CD7, are rarely expressed.

One study found that the combination of CD19 and CD34 is most reliable in predicting t(8;21) (9). Using the cutoff of 10% for CD19 and 35% for CD34, this combination correctly classified 92 of 93 AML cases with t(8;21).

By using immunohistochemistry, the most exciting recent finding is the presence of the PAX5 protein (B-cell-specific activator protein) only in AML cases with t(8;21), but it was positive in only one third of the cases studied (15). In some t(8;21) cases without a positive immunohistochemical

staining for PAX5, up-regulation of PAX5 transcript was identified by real-time reverse transcription–polymerase chain reaction (RT-PCR) studies (15). PAX5 is the master regulator of B-lymphopoiesis through activation of B-cell-specific genes, including CD19 and CD79a (16,17). Therefore, it is not unexpected that CD19 and CD79a are also expressed, though in lower frequency, in PAX5-positive cases. As there is no CD19 monoclonal antibody for immunohistochemistry, the identification of these two B-cell markers, PAX5 and CD79a, is most useful in surgical pathology.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is usually more practical than immunohistochemistry in the study of blood and bone marrow specimens and is capable in identifying this special subtype of AML by showing CD19 and CD34 in addition to myeloid markers. However, immunohistochemistry is most helpful in diagnosing myeloid sarcoma derived from this subtype of AML by using myeloid markers together with PAX5 and CD79a.

Molecular Genetics

Molecular characterization has demonstrated that t(8;21) represents the fusion of the RUNX1 gene on chromosome 21q22 with the RUNX1T1 gene on chromosome 8q22. The RUNX1 gene is also called core binding factor protein a (CBFa), AML1, and FEBP2. RUNX1T1 is also called ETO and MTG8.

The RUNX1 gene encodes the CBFa protein, which forms a heterodimer with CBFb that plays an important role in normal hematopoietic differentiation (9,18,19). CBF also cooperates with other basic transcription factors in activating a set of hematopoietic specific genes. The RUNX1T1 gene is the mammalian homolog of the *Drosophila* gene *nervy*, a transcriptional regulator with yet unknown biologic function (9).

RUNX1-RUNX1T1 encodes a fusion transcript with a primary inhibitory role in normal hematopoietic differentiation. It regulates the expression of both RUNX1 target and non-RUNX1 target genes via its interaction with various transcription regulators (18). However, t(8;21) alone cannot induce leukemia. Additional mutations are necessary for the development of AML (18).

Translocation (8;21) is one of the most common AML cytogenetic abnormalities, occurring in 7% to 8% of adult cases and 11.7% of pediatric cases (9). As mentioned before, most cases present with M2 morphology. The incidence ranges from one third to 46% for M2 cases with an abnormal karyotype (20,21). A study of childhood leukemia in a single institute showed that 82% of AML cases with t(8;21) were M2 cases and that 23% had granulocytic sarcoma (22). A German study revealed that, among AML cases with t(8;21), 12.5% were M2, 1.7% M1, 0.09% M3 to M7, and 0% M0 (23). Other studies reported t(8;21) in cases of M1, M4, M4Eo, chronic myeloid leukemia, and myelodysplastic syndrome (4,5, 24–28). However, in the light of the World Health Organization (WHO) definition, those cases of myelodysplastic syndrome should probably be classified as AML cases (1).

The positive rate for this abnormality is higher when studied with molecular biology techniques (4,5,29). In a study of 64 patients, Andrieu et al. (5) detected 8% cases

TABLE 6.5.1

Special Morphologic Features in AML with t(8;21)(q22;q22)

1. FAB AML with maturation (AML-M2) morphology, including type 3 blasts in some cases
2. Abundant Auer rods in mature and immature myeloid cells
3. Salmon-colored granules and a rim of basophilic cytoplasm in myeloid cells
4. Pseudo-Chediak–Higashi anomaly
5. Cells with pink, waxy cytoplasmic globules
6. Large blasts with prominent Golgi area
7. Cytoplasmic vacuoles
8. Bone marrow eosinophilia (>5%)

with t(8;21) by karyotyping, but 16% of cases showed RUNX1-RUNX1T1 by an RT-PCR assay. In a survey from the Cancer and Leukemia Group B, RUNX1-RUNX1T1 was detected in other abnormal karyotypes, such as t(8;10)(q22;q26) and t(1;10;8)(p22;p13;q22) (29). Other complex translocations such as t(8;12;21) and t(8;17;21) have been reported (30,31). By the FISH method, the presence of RUNX1-RUNX1T1 in other karyotypes was found to be the result of cryptic insertion (29,32). The abnormality can be the RUNX1 gene inserts into 8q22 or RUNX1T1 into 21q22. The RUNX1-RUNX1T1 fusion product can be detected by RT-PCR even when the patient is in remission for as long as 8 years (20).

Mutations of KIT are common (12% to 47%) in this subtype of AML (33,34). In adults, KIT mutations in exons 8 and 17 are associated with a worse prognosis (35). In pediatric patients with core-binding factor (CBF)-AML (t[8;21] and inv[16]), KIT mutation was detected in 19% (38 of 203 patients) cases with comparable percentages involving exon 8 and exon 17 (36). However, rates of complete remission, overall survival, disease-free survival (DFS), or relapse were not significantly different for patients with or without KIT mutation. In a gene expression profiling study, KIT mutated CBF-AML cases were characterized by deregulation of genes belonging to the NFκB signaling complex suggesting impaired control of apoptosis (37). Another study suggested that microRNA (MIR)-222/221 represents the link between CBF and KIT (38). RUNX1-RUNX1T1 fusion protein binds the MIR-222/221 promoter and induces transcriptional repression of MIR-222/221-LUC receptor. As a result, the KIT receptor is up-regulated (38).

Mutations of FLT3-ITD and FLT3-TKD are uncommon with a frequency of 2% to 9% and 2% to 7%, respectively, in t(8;21) patients (34). The clinical significance of these mutations remains to be established (34). JAK2-V617F mutation is also infrequently seen in CBF-AML (34,39). However, DFS of CBF-AML patients with JAK2 mutations was significantly worse than DFS of patients without these mutations in one study (34). Secondary cooperating mutations of KRAS or NRAS are common (30%) in pediatric cases (8). RAS mutations are present in 8% to 11% of t(8;21) AML patients, but these mutations have not been correlated with clinical outcome (34).

In a large number of patients, additional chromosome abnormalities or complex translocation are identified (1,9). The most common cytogenetic abnormalities are loss of sex chromosome and partial deletion of the long arm of chromosome 9 (8,34). In general, the presence of additional cytogenetic aberrations does not seem to affect the prognosis (35), but recent studies suggested that TLE1 and TLE4 genes, which are candidate tumor suppressors, were invariably lost in t(8;21) patients with del(9q) (34).

The immunophenotypic and molecular genetic features of AML with t(8;21) are summarized in Table 6.5.2.

Clinical Manifestation

AML-M2 with t(8;21) is frequently seen in patients younger than 60 years, particularly children. This special chromosomal abnormality is not usually seen in elderly AML patients, with a frequency <2% (9). The majority of cases with t(8;21) occur in primary de novo AML cases. However, it is also present occasionally in secondary AML patients.

TABLE 6.5.2

Immunophenotypic and Molecular Genetic Features in Acute Myeloid Leukemia (AML) with t(8;21)(q22;q22)

1. CD19 (>10%) and CD34 (>35%) in a myeloid population
2. Presence of CD56 predicting unfavorable prognosis with potential development of myeloid sarcoma
3. Demonstration of PAX5 and CD79a by immunohistochemistry
4. Karyotype: t(8;21)(q22;q22)
5. Molecular biology: RUNX1-RUNX1T1

The clinical symptoms of AML with t(8;21) are similar to those seen in other acute leukemias, namely, bone marrow failure. A particular frequent presentation is granulocytic (myeloid) sarcoma, which involves solid organs, and the bone marrow may show <20% of myeloblasts (1). In one study, the complete remission rate in patients with myeloid sarcoma was 50% as compared to 94% in those without myeloid sarcoma (7). These patients also had a significantly shorter survival.

In general, t(8;21) confers a favorable prognosis; adult patients usually have a good response to chemotherapy, with high remission rates and long-term disease-free survival when treated with high dose cytarabine (1). Pediatric patients, however, have a much less favorable response than adult patients do (25).

Besides age and the presence of myeloid sarcoma, immunophenotype also affects the prognosis. The presence of CD56 usually relates to inferior disease-free survival (10). This study also found that myeloid sarcoma was present exclusively in cases with CD56 expression. The association of leukocyte count and prognosis in this entity is inconclusive. However, the so-called white blood cell (WBC) index, calculated as the product of WBC count by the percentage of blasts in the bone marrow, was a more reliable and independent predictor for relapse-free survival (40).

REFERENCES

1. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukemia with recurrent genetic abnormalities. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissue*. Lyon, France: IARC Press; 2001:81–87.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:620–624.
3. Willman CL. Acute leukemias: a paradigm for the integration of new technologies in diagnosis and classification. *Mod Pathol*. 1999;12:218–228.
4. Nucifora G, Dickstein JL, Torbenson V, et al. Correlation between cell morphology and expression of the AML1/ETO chimeric transcript in patients with acute myeloid leukemia without the t(8;21). *Leukemia*. 1994;8:1533–1538.
5. Andrieu V, Radford-Weiss I, Troussard X, et al. Molecular detection of t(8;21)/AML1-ETO in AML M1/M2: correlation

- with cytogenetics, morphology and immunophenotype. *Br J Haematol*. 1996;92:855–865.
6. Tallman MS, Hakimian D, Shaw JM, et al. Granulocytic sarcoma is associated with the 8;21 translocation in acute myeloid leukemia. *J Clin Oncol*. 1993;11:690–697.
 7. Byrd JC, Weiss RB, Arthur DC, et al. Extramedullary leukemia adversely affects hematologic complete remission rate and overall survival in patients with t(8;21) (q22;q22): results from Cancer and Leukemia Group B 8461. *J Clin Oncol*. 1997;15:466–475.
 8. Arber DA, Brunning RD, Le Beau MM, et al. Acute myeloid leukemia with t(8;21)(q22;q22); RUNX1-RUNX1T1. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:110–111.
 9. Ferrara F, Vecchio LD. Acute myeloid leukemia with t(8;21)/AML1/ETO: a distinct biological and clinical entity. *Haematologica*. 2002;87:306–319.
 10. Baer MR, Stewart CC, Lawrence D, et al. Expression of the neural cell adhesion molecule CD56 is associated with short remission duration and survival in acute myeloid leukemia with t(8;21)(q22;q22). *Blood*. 1997;90:1643–1648.
 11. Wang YY, Zhou GB, Yin T, et al. AML1-ETO and C-KIT mutation/overexpression in t(8;21) leukemia: implication in stepwise leukemogenesis and response to Gleevec. *Proc Natl Acad Sci U S A*. 2005;102:1104–1109.
 12. Ferrara F, DiNoto R, Annunziata M, et al. Immunophenotypic analysis enables the correct prediction of t(8;21) in acute myeloid leukaemia. *Br J Haematol*. 1998;102:444–448.
 13. Hurwitz CA, Raimondi SC, Head D, et al. Distinctive immunophenotypic features of t(8;21)(q22;q22) acute myeloid leukemia in children. *Blood*. 1992;80:3182–3188.
 14. Basso G, Buldini B, De Zen L, et al. New methodologic approaches for immunophenotyping acute leukemia. *Haematologica*. 2001;86:675–692.
 15. Tiacci E, Pileri S, Orieth A, et al. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. *Cancer Res*. 2004;64:7399–7404.
 16. Kozmik Z, Wang S, Dorfler P, et al. The promoter of the CD19 gene is a target for the B-cell specific transcription factor BSAP. *Mol Cell Biol*. 1992;12:2662–2672.
 17. Fitzsimmons D, Hodsdon W, Wheat W, et al. Pax-5 (BSAP) recruits Ets protooncogene family proteins to form functional ternary complexes on a B-cell-specific promoter. *Genes Dev*. 1996;10:2198–2211.
 18. Peterson LF, Zhang DE. The 8;21 translocation in leukemogenesis. *Oncogene*. 2004;23:4255–4262.
 19. Roumier C, Fenaux P, Lafage M, et al. New mechanisms of AML1 gene alteration in hematological malignancies. *Leukemia*. 2003;17:9–16.
 20. Nucifora G, Rowley JD. The AML and ETO genes in acute myeloid leukemia with a t(8;21). *Leuk Lymphoma*. 1994;14:353–362.
 21. Caligiuri MA, Strout MP, Gilliland DG. Molecular biology of acute myeloid leukemia. *Semin Oncol*. 1997;24:399–408.
 22. Rubnitz JE, Raimondi SC, Halbert AR, et al. Characteristics and outcome of t(8;21)-positive childhood acute myeloid leukemia: a single institution's experience. *Leukemia*. 2002;16:2072–2077.
 23. Klaus M, Haferlach T, Schnittger S, et al. Cytogenetic profile in de novo acute myeloid leukemia with FAB subtypes M0, M1, and M2: a study based on 652 cases analyzed with morphology, cytogenetics, and fluorescence in situ hybridization. *Cancer Genet Cytogenet*. 2004;155:47–56.
 24. Downing JR, Head DR, Curchi-Brent MG, et al. An AML1/ETO fusion transcript is consistently detected by RNA-based polymerase chain reaction in acute myelogenous leukemia containing the (8;21)(q22;q22) translocation. *Blood*. 1993;81:2860–2865.
 25. Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. *Blood*. 1995;86:1–14.
 26. Kojima K, Omonot E, Hara M, et al. Myelodysplastic syndrome with translocation (8;21): a distinct myelodysplastic syndrome entity or M2-acute myeloid leukemia with extensive myeloid maturation? *Ann Hematol*. 1998;76:279–282.
 27. Mathew S, Shurtleff S, Ribeiro RC, et al. A complex variant t(8;21) involving chromosome 3 in a child with acute myeloblastic leukemia with eosinophilia (AML M4Eo). *Leuk Lymphoma*. 2003;44:183–187.
 28. Yan CC, Medeiros LJ, Glassman AB, et al. t(8;21)(q22;q22) in blast phase of chronic myelogenous leukemia. *Am J Clin Pathol*. 2004;121:836–842.
 29. Mrozek K, Prior TW, Edwards C, et al. Comparison of cytogenetic and molecular genetic detection of t(8;21) and inv(16) in a prospective series of adults with de novo acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2001;19:2482–2492.
 30. Farra C, Awwad J, Valent A, et al. Complex translocation (8;12;21): a new variant of t(8;21) in acute leukemia. *Cancer Genet Cytogenet*. 2004;155:138–142.
 31. Miyagi J, Kakazu N, Masuda M, et al. Acute myeloid leukemia (FAB-M2) with a masked type of t(8;21) translocation revealed by spectral karyotyping. *Int J Hematol*. 2002;76:338–343.
 32. Urioste M, Martinez-Ramirez A, Cigudosa JC, et al. Identification of ins(8;21) with AML1/ETO fusion in acute myelogenous leukemia M2 by molecular cytogenetics. *Cancer Genet Cytogenet*. 2002;133:83–86.
 33. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21); a Cancer and Leukemia Group B study. *J Clin Oncol*. 2006;24:3904–3911.
 34. Mrozek K, Marcucci G, Paschka P, et al. Advances in molecular genetics and treatment of core-binding factor acute myeloid leukemia. *Curr Opin Oncol*. 2008;20:711–718.
 35. Arber DA, Heerema-McKenney A. Acute myeloid leukemia. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:672–697.
 36. Pollard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood*. 2010;115:2372–2379.
 37. Luck SC, Russ AC, Du J, et al. KIT mutations confer a distinct gene expression signature in core binding factor leukaemia. *Br J Haematol*. 2010;148:925–937.
 38. Brioschi M, Fischer J, Cairoli R, et al. Down-regulation of microRNAs 222/221 in acute myelogenous leukemia with deranged core-binding factor subunits. *Neoplasia*. 2010;12:866–876.
 39. Iwanaga E, Nauri T, Matsuno N, et al. A JAK2-V617F activating mutation in addition to KIT and FLT3 mutation is associated with clinical outcome in patients with t(8;21)(q22;q22) acute myeloid leukemia. *Hematologica*. 2009;94:433–435.
 40. Dombret H, N'Guyen S, Leblanc T. Prognostic factors in t(8;21) acute myeloid leukemia (AML): an overview from the French AML Intergroup (LAME, GOELAM, BGMT, ALFA, SFGM) [abstract]. *Hematol J*. 2001;1(Suppl 1):196a(abst).

CASE 6

Acute Myeloid Leukemia with inv(16) (p13q22) or t(16;16)(p13;q22)

CASE HISTORY

A 52-year-old man presented with shortness of breath, fatigue, and hypersomnolence for 5 months. He was transferred from another hospital for evaluation of likely leukemia. Physical examination on admission was unremarkable except for pale conjunctivae, ecchymosis on the right hand, and a palpable cervical lymph node. There was no hepatosplenomegaly. Hematology workup showed a total leukocyte count of 38,400/mL with 57% blasts, 3% neutrophils, 32% monocytes, and 8% lymphocytes. A bone marrow biopsy revealed 51% myeloblasts, 24% monoblasts, 13% myeloid cells of various stages, 7% eosinophils, 8% monocytes, and 1.5% erythroid elements. He was then treated with cytarabine and daunorubicin. The clinical course was complicated with neutropenic fever, *Clostridium difficile* colitis, and possible candidiasis in the liver and spleen, as demonstrated by computed tomography imaging. All the complications were gradually gotten under control by

antibiotic therapy and transfusion. The second bone marrow biopsy demonstrated no leukemic cells. The patient was discharged 1 month after admission.

FLOW CYTOMETRIC FINDINGS

Bone marrow: Myeloid cells: Myeloperoxidase 97%, CD13-CD33 96%, CD14 34%, CD13-CD33/CD7 0%, and HLA-DR 88%. Stem cell markers: CD34 85%, CD117 96% (Fig. 6.6.1).

CYTOCHEMICAL FINDINGS

In the bone marrow, the myeloperoxidase stain was positive in both myeloblasts and monoblasts as well as the maturing myelomonocytic cells. The chloroacetate esterase stain identified about 70% myeloid cells, and the α -naphthyl butyrate esterase stain identified 30% monocytic cells (Fig. 6.6.2).

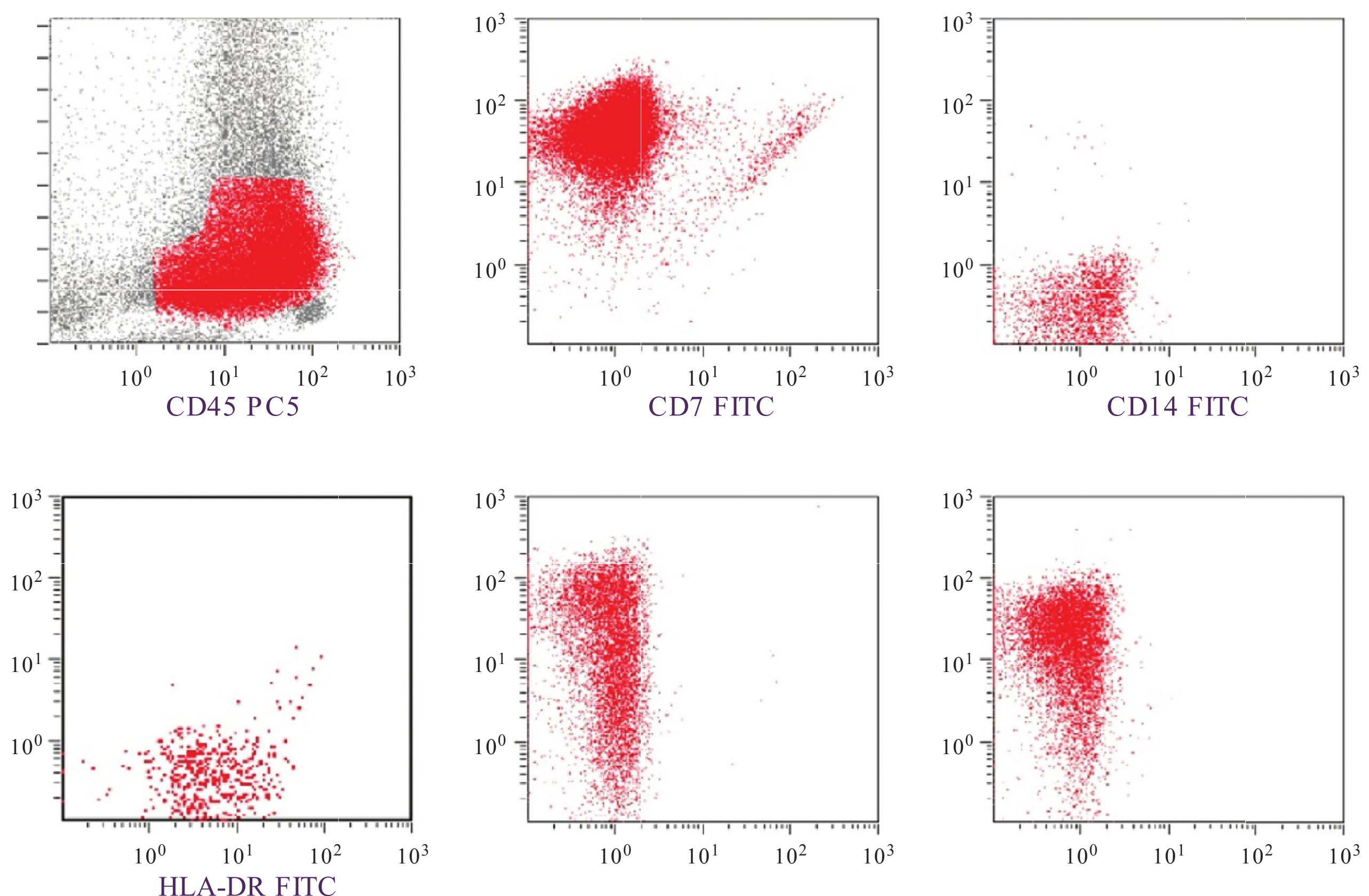


FIGURE 6.6.1 Flow cytometric analysis of bone marrow shows positive reactions with CD13, CD33, CD7, CD14, HLA-DR, CD34, and CD117. SS, side scatter; PE, phycoerythrin, RD1, rhodamine; FITC, fluorescein isothiocyanate.

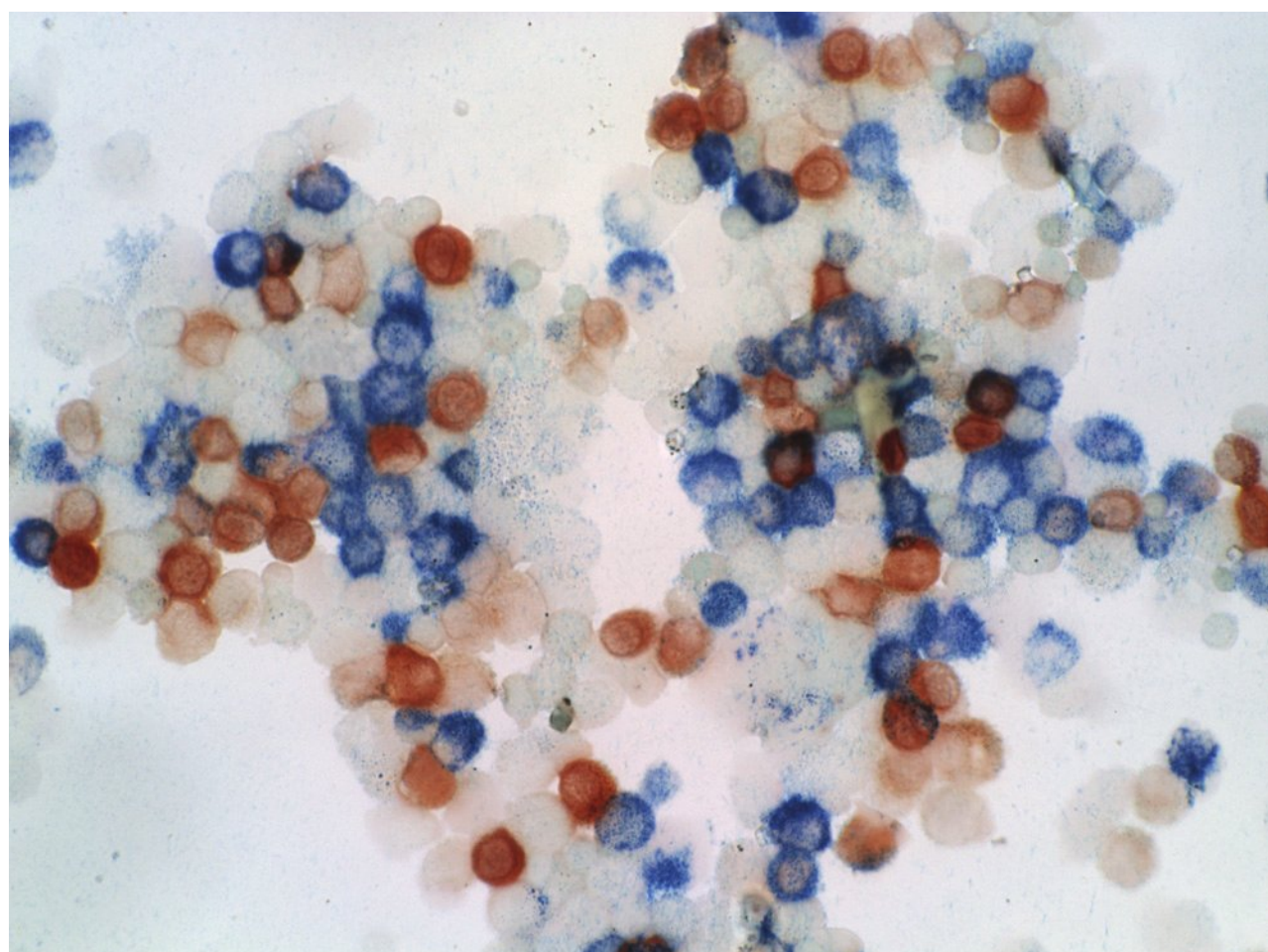
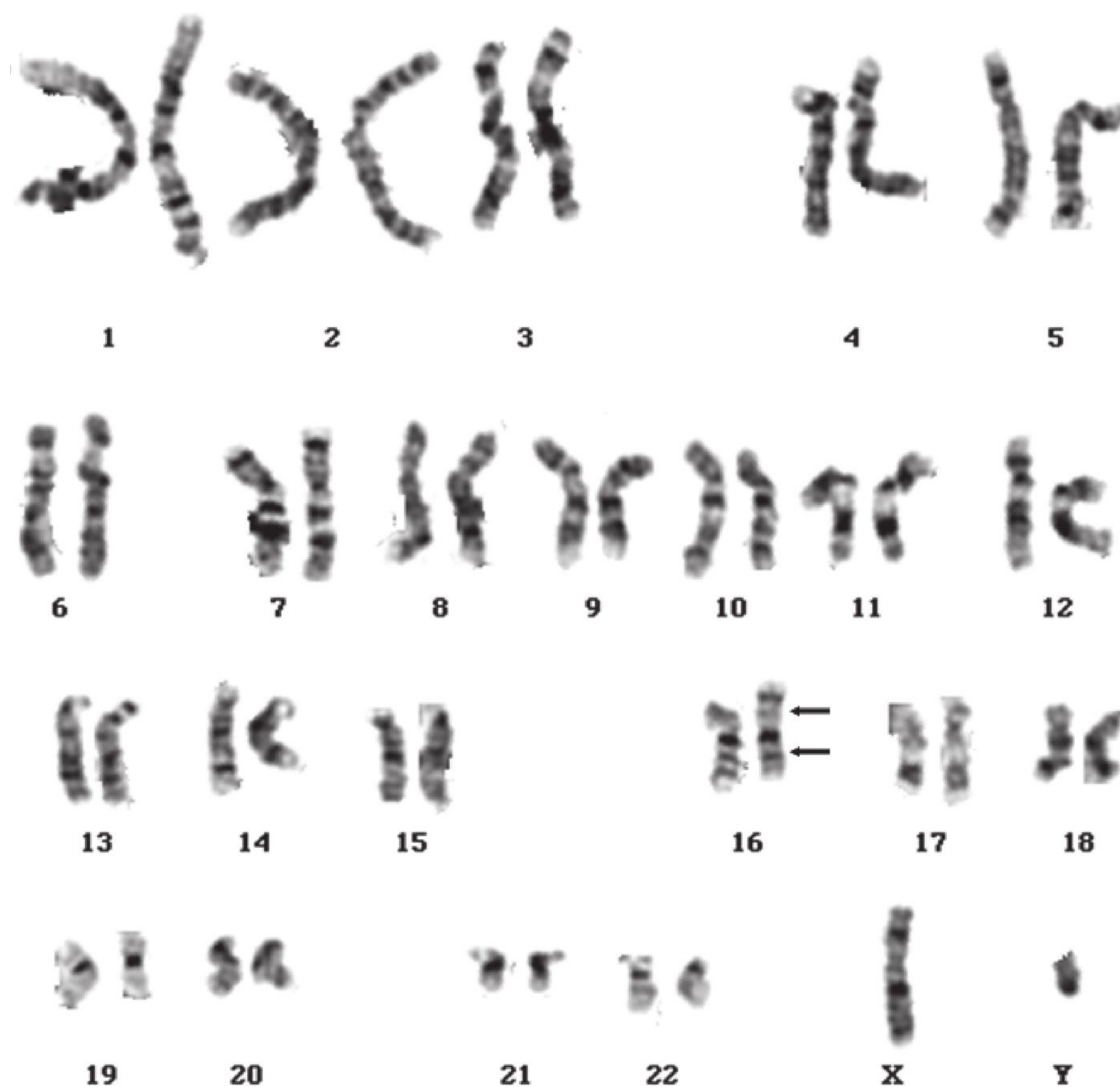


FIGURE 6.6.2 Combined esterase stain of the bone marrow cytospin shows chloroacetate esterase stain (blue) of the myeloid cells and α -naphthyl butyrate esterase stain (brown) of the monocytoid cells. 40 $\times$ magnification.

CYTOGENETIC FINDING

The bone marrow showed a karyotype as following: 46,XY, del(7)(q22q34), inv(16)(p13q22) [8]/48,idem, +9, +22 [3]/46, XY [9] (Fig. 6.6.3 shows only inv(16)(p13q22)).

FIGURE 6.6.3 Karyotype of the bone marrow reveals inversion of chromosome 16 (arrows). (Courtesy of Peter Papenhausen, Ph.D., LabCorp of America Cytogenetics Department.)



DISCUSSION

Acute myeloid leukemia (AML) with inv(16)(p13q22) or t(16;16)(p13;q22) is seen predominantly in cases of acute myelomonocytic leukemia with eosinophilia (AML M4Eo). It accounts for approximately 5% of all patients with AML and 20% of AML-M4 cases (1). However, this karyotype has also been encountered in other subtypes of AML, chronic myeloid leukemia, and myelodysplastic syndromes (2,3). In the study by Mitelman and Heim (4), 206 of the total 241 inv(16) cases were diagnosed as M4 subtype. However, this aberration was also demonstrated in 17 M2 cases, 10 M5 cases, and 1 to 3 cases each of M1, M6, and M7. M0, M1, M2, M4, and M5 have been reported in other studies (2,5,6). In one study, approximately 10% of M4 cases without eosinophilia showed this karyotype (7). Inv(16) has also been reported in several cases of chronic myeloid leukemia with blast crisis, in which bone marrow eosinophilia was also observed (2).

In the current case, the bone marrow showed approximately 70% myeloid cells and 30% monocytic cells, as identified by cytochemical stain, a ratio that is roughly equivalent to the morphologic differential count. Flow cytometric analysis also showed positive myelomonocytic markers with high percentages of CD34 and CD117, which is consistent with acute myelomonocytic leukemia. Bone marrow eosinophil count is >5%; therefore, it fulfills the definition of M4Eo. Cytogenetic study of the bone marrow shows a

complex karyotype including inv(16)(p13q22); thus, it is considered to be AML with inv(16).

Morphology and Cytochemistry

The French-American-British definition of M4 is that the myeloid or monocytic component, whichever is the majority, should not be >80% of the nonerythroid population in the bone marrow (8) (Figs. 6.6.4 and 6.6.5). In addition, the percentage of the blast, which is composed of myeloblasts and monoblasts, should be >30%. The cell lineage identification is based on cytochemical stains: myeloperoxidase, specific esterases, and nonspecific esterases (see Case 8). The World Health Organization system lowers the cutoff of the blast count to 20%. However, in AML with inv(16) or t(16;16), the blast count can be <20% and it is still acceptable for AML (9,10). The peripheral blood may show monocytosis but usually no eosinophilia (Fig. 6.6.6).

The eosinophils in these cases usually show all stages of maturation (9). Abnormal eosinophilic granules are often seen in the myelocyte and promyelocyte stages. In those eosinophils, there are mixed eosinophilic and basophilic, or purple-violet granules (Fig. 6.6.7). These granules are larger than those in normal eosinophils of the same stage, and sometimes the granules are so numerous that they obscure the nucleus.

Cytochemical stains of these abnormal eosinophils also differ from those of normal eosinophils. Unlike their normal counterparts, these eosinophils react with chloroacetate esterase and periodic acid-Schiff (11). The basophilic granules are positive for myeloperoxidase and negative for toluidine blue; these reactions are opposite to the reactions seen in normal basophils (11). Ultrastructurally, the abnormal eosinophils are characterized by the absence of well-formed central crystalloids in the cytoplasmic granules (11). Eosinophils contain high levels of lysozyme, so that an elevated serum lysozyme value cannot be used as a criterion for monocytic differentiation when eosinophilia is present in an AML case (12). For instance, M2 with eosinophilia may have a high lysozyme concentration in the blood.

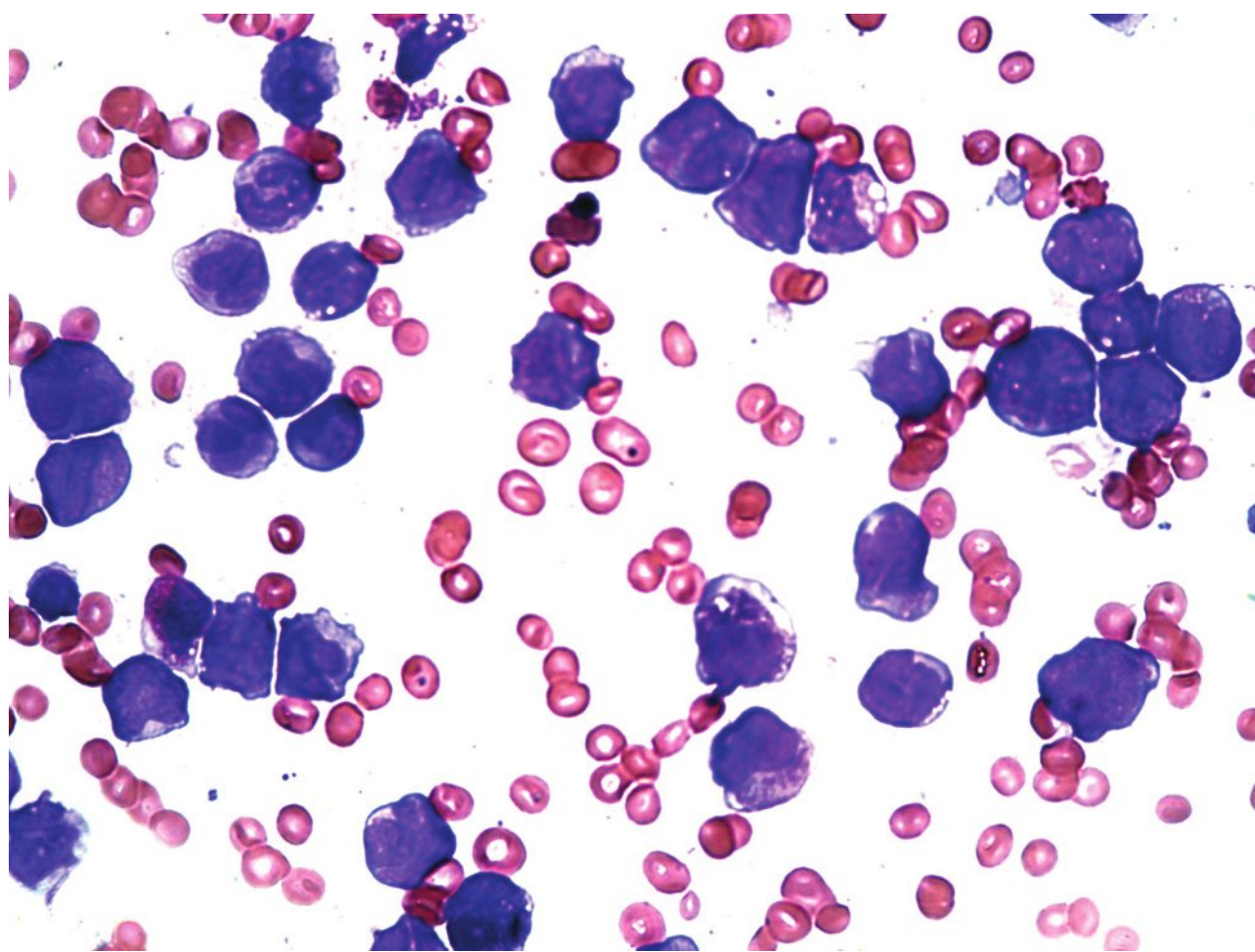


FIGURE 6.6.4 Bone marrow aspirate shows various developmental stages of myelomonocytic cells. Wright-Giemsa, 60× magnification.

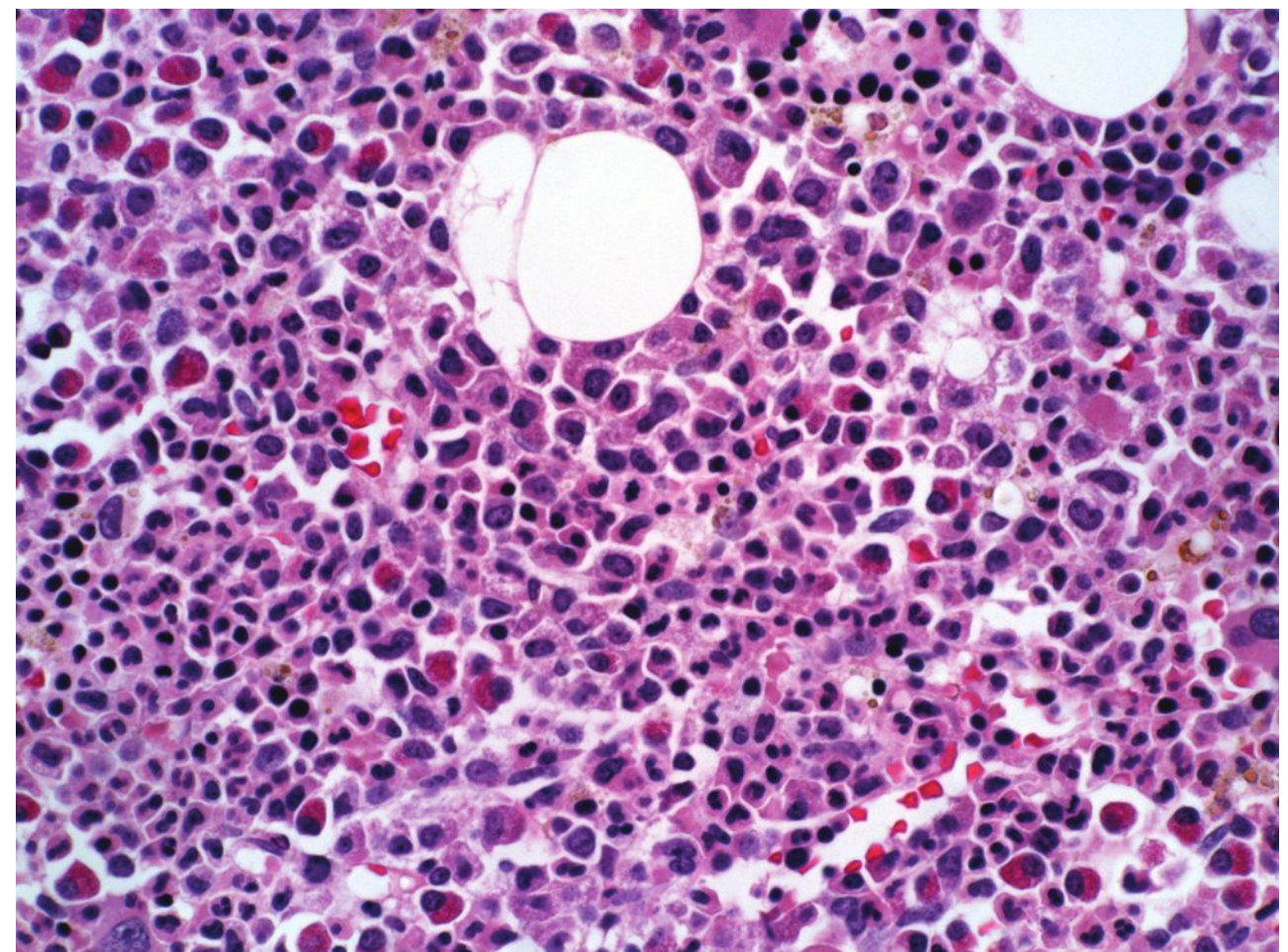


FIGURE 6.6.5 Bone marrow core biopsy reveals hypercellular marrow composed of immature myelomonocytic cells. Eosinophilia is evident. Hematoxylin and eosin, 40× magnification.

Whether the eosinophils are leukemic cells in M4Eo cases is controversial. There was one report showing inv(16) in the eosinophils of an M4Eo case (13), but this result has not been confirmed by other studies. Eosinophilia can sometimes behave as a preleukemic syndrome leading to M4 (14,15).

A study of 21 cases showed that dysplasia is a prominent feature in AML with inv(16), which is associated with a significantly higher proliferation rate, as demonstrated by immunohistochemical staining with Mib-1 (Ki-67) (16). However, apoptotic rate in M4Eo is similar to that in other AML subtypes.

Immunophenotype

As in other types of AML, the blasts of M4Eo express CD13, CD15, CD33, myeloperoxidase, and HLA-DR. In addition, monocytic component is represented by one or more of

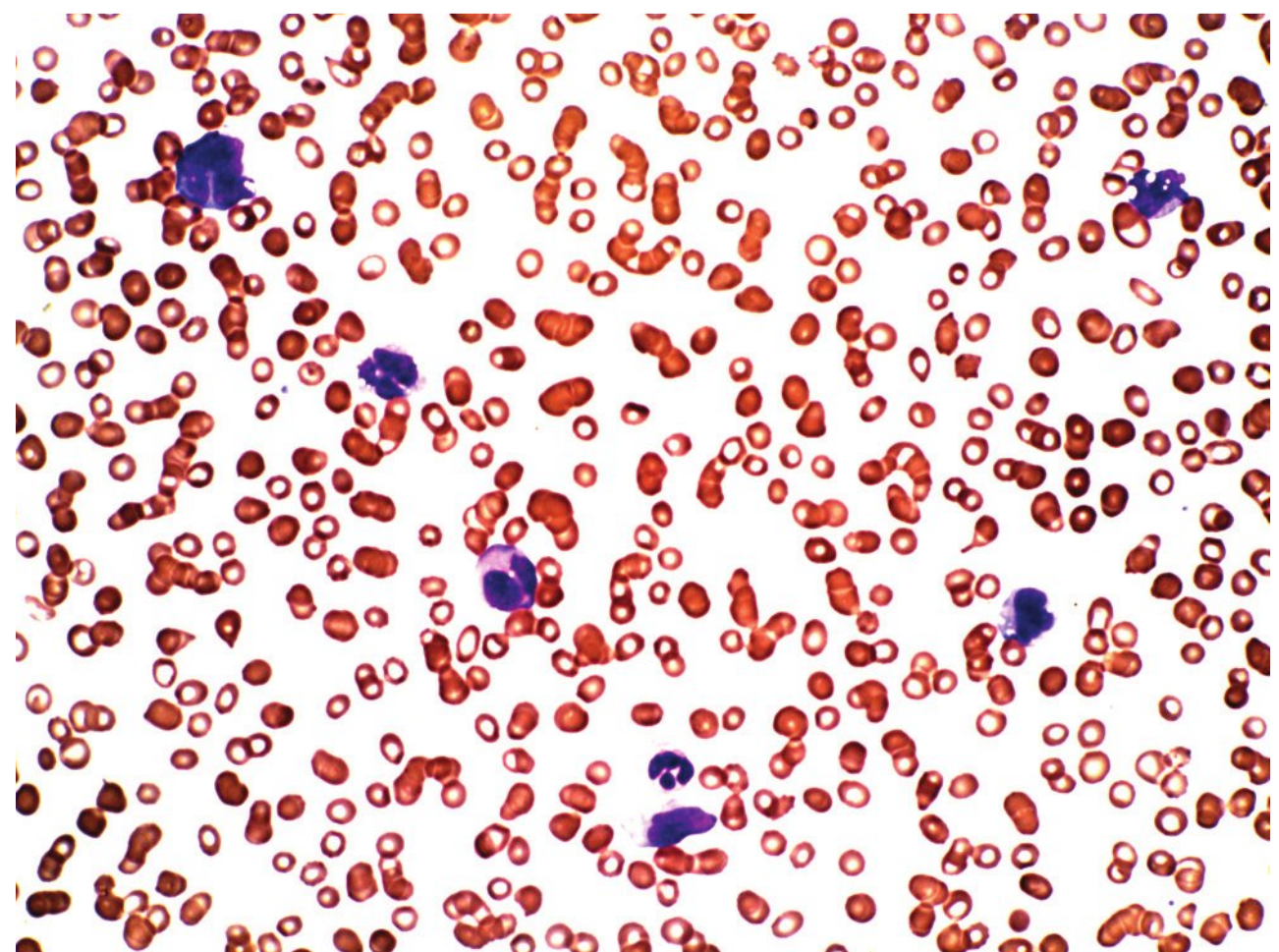


FIGURE 6.6.6 Peripheral blood smear shows monocytosis. Wright-Giemsa, 60× magnification.

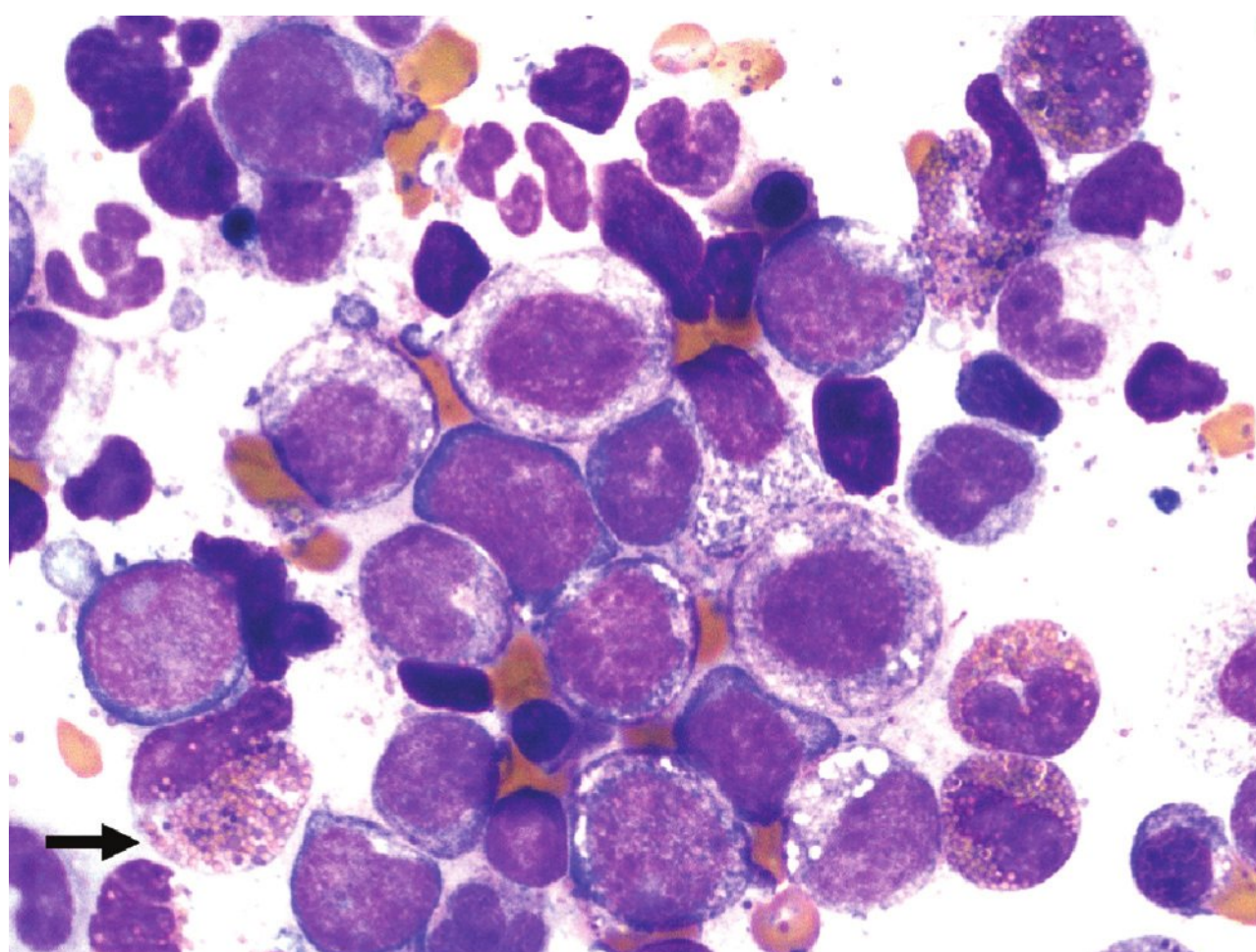


FIGURE 6.6.7 Bone marrow aspirate reveals immature myelomonocytic cells with the presence of basophilic granules in a few immature eosinophils (arrow). 100× magnification.

the monocytic markers, such as CD4, CD11b, CD11c, CD14, CD36, CD64, and lysozyme (10,17). The malignant nature of the leukemic cells is identified by the presence of CD34 and CD117 (18). These markers, however, can be demonstrated in acute myelomonocytic leukemia with or without eosinophils. The coexpression of CD2 with myeloid markers is a clue to the diagnosis of this subtype of AML, but it is not entirely specific (17). In cell culture of two M4Eo cases, the addition of CD2 antibodies caused reduced cell proliferation. It is therefore assumed that the CD2 molecule may stimulate the proliferation of the leukemic cells and cause a high leukocyte count in M4Eo (17).

Terminal deoxynucleotidyl transferase (TdT) expression has been reported in two studies (17,19). The TdT-positive cells were seen exclusively in the CD34+ CD14- subpopulation (17).

Comparison of Flow Cytometry and Immunohistochemistry

Immunohistochemical staining may demonstrate myeloperoxidase, lysozyme, CD68, CD34, and CD117. In comparison, flow cytometry is preferred to immunohistochemistry, because the histogram of flow cytometry may demonstrate the heterogeneous cell populations with more markers.

Molecular Genetics

In M4Eo cases, three abnormal karyotypes involving chromosome 16 can be demonstrated: inv(16)(p13q22), t(16;16)(p13;q22), and del(16)(q22). Most cases carry the inv(16) karyotype, and del(16q) is least frequently seen. One study showed marked differences in survival and remission duration between the inv(16) or t(16;16) patients and those with del(16q) (3).

Cloning of the 16p and 16q breakpoints identified the core binding factor (CBF)b and smooth muscle myosin heavy chain (MYH)11 genes located at 16q22 and 16p13, respectively (2). The MYH11 gene codes for a smooth

muscle MYH gene. The CBFb gene, also known as polyoma enhancer binding protein 2b, codes for the b subunit of CBF, a heterodimeric transcription factor. The a subunit of CBF is identical to the RUNX1 gene, which is involved in t(8;21) translocation (see Case 5). In vitro analysis showed that the murine CBFb gene formed a heterodimeric complex with CBFa, thus stabilizing its interaction with DNA (7). As both t(8;21) and inv(16) or t(16;16) are characterized by the disruption and transcriptional deregulation of genes encoding subunits of the CBF, which is involved in the regulation of normal hematopoiesis, these two types of AML are called CBF AML (20). However, the fusion gene CBFb-MYH11 may not be sufficient for leukemogenesis; additional genes may be involved in the pathogenesis (21).

The CBFb-MYH11 fusion gene can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR). RT-PCR studies demonstrated the existence of marked molecular heterogeneity in terms of breakpoint location, and eight types of fusion transcripts have been reported, with the A type being most common (88%) (2). A J type has been reported recently (22). In comparison with the A type, the rare fusion transcripts are associated with more frequent therapy-related M4Eo, additional chromosomal rearrangements, and lower white cell count (23). These patients usually show <5% or total absence of eosinophils, so that they may be misdiagnosed as other subtypes of AML (23). Immunophenotypically, these patients show weaker expression of CD2, CD13, CD33, and CD90 (23).

Cytogenetic detection of inversions and small deletions of chromosome 16 by standard karyotyping can be difficult (7). The detection of such cytogenetic abnormalities is affected by the ability to obtain adequate metaphases and the coexistence of normal metaphases (7). Several studies have demonstrated the higher sensitivities with fluorescence in situ hybridization (24–26) and RT-PCR (2,7) techniques than with the conventional karyotyping. One report showed no cytogenetic abnormality by conventional karyotyping and FISH in a case of M4Eo, but CBFb-MYH11 was identified by RT-PCR (27).

Cases with inv(16) are often accompanied by additional abnormalities with a frequency as high as 50% in one study (3). However, the presence of additional changes does not affect the response to therapy or the survival (28,29). Only one report suggested that coexistence of inv(16) and partial deletion of the CBFb gene could be associated with unfavorable prognosis (26). The most commonly observed secondary cytogenetic aberrations include +22, +8, (10% to 15% each) and del(7q) or +21 (□5%) (10). Trisomy 21 is fairly specific for inv(16) patients as it is seldom seen in other subtypes of AML, while trisomy 8 is commonly seen in other AML cases as well (10).

The most common gene mutation is demonstrated in KIT gene, which is detected in 22% to 38% of patients with inv(16)/t(16;16) (30). This mutation carries an unfavorable prognosis in general (31). However, in a pediatric series, rates of complete remission, overall survival, disease-free survival or relapse were not significantly different for patients with or without KIT mutations (32).

TABLE 6.6.1

Salient Features for Laboratory Diagnosis of AML with inv(16)(p13q22) or t(16;16)(p13;q22)

1. Karyotype: inv(16)(p13q22), t(16;16)(p13;q22), or del(16)(q22)
2. Molecular characterization by FISH or RT-PCR: CBFb-MYH11 fusion gene
3. Presence of >20% myeloblasts and monoblasts in the bone marrow; <20% blasts is acceptable when typical karyotype or molecular pattern is identified
4. Bone marrow contains myeloid and monocytoid cells with the minority cell component >20%
5. Bone marrow contains >5% eosinophils. Absence of eosinophilia is acceptable. Eosinophils are abnormal in cytoplasmic granules and cytochemical stains.
6. Immunophenotype: Flow cytometry may demonstrate myelomonocytic markers (CD13, CD33, myeloperoxidase, CD14, CD11b, CD11c) and immature cell markers (CD34 and CD117). One special marker is CD2, a T-cell marker that is coexpressed with myeloid markers.

FISH, fluorescence in situ hybridization; RT-PCR, reverse transcription–polymerase chain reaction; CBF, core binding factor; MYH, smooth muscle myosin heavy chain gene.

Gene expression profiling study showed that KIT mutated CBF-AML cases were characterized by deregulation of genes belonging to the NFκB signaling complex, suggesting impaired control of apoptosis (33).

FLT3-ITD mutation has been found in 0% to 7% and FLT3-TKD in 6% to 24% of inv(16) patients, but the clinical significance of these mutations is still unclear (30). NRAS and KRAS gene mutations are common among inv(16)/t(16;16) patients, being detected in over one third of cases studied (30), but clinical correlation with these mutations has not been established (30).

The salient features for laboratory diagnosis of AML with inv(16) or t(16;16) are summarized in Table 6.6.1.

Clinical Manifestations

AML with inv(16) or t(16;16) has been reported in all age groups, but it is predominantly seen in young patients. CBF AML accounts for up to 20% of young adult cases of de novo AML (25). In a study of 43 pediatric cases of AML in Hong Kong, 5 patients were found to have this abnormality (34).

AML with inv(16) or t(16;16) is usually associated with a favorable prognosis in terms of complete remission and the duration of remission and survival when compared with other AML M4 cases with similar treatment (7). There is no clinical difference between patients with inv(16) and those with t(16;16) (2,3,35). However, patients with del(16q) are different; the outcome of those patients was

not better than that of other AML M4 patients in one study (3). In addition, del(16q) cases lack relapse in the central nervous system (CNS) and have lower incidence of eosinophilia and M4 subtype (3).

Clinical symptoms are similar to those seen in other M4 cases. Specific features of AML with inv(16) include a high leukocyte count, hepatosplenomegaly, and high incidence of CNS leukemia, manifested as leptomeningeal disease and intracerebral myeloblastomas (17). Myeloid sarcoma may be present at initial diagnosis or at relapse (9).

REFERENCES

1. Larson RA, Williams SF, Le Beau MM, et al. Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) and t(16;16) has a favorable prognosis. *Blood*. 1986;68:1242–1249.
2. Liu PP, Hajra A, Wijmenga C, et al. Molecular pathogenesis of the chromosome 16 inversion in the M4E0 subtype of acute myeloid leukemia. *Blood*. 1995;85:2289–2302.
3. Marlton P, Keating M, Kantarjian H, et al. Cytogenetic and clinical correlates in AML patients with abnormalities of chromosome 16. *Leukemia*. 1995;9:965–971.
4. Mitelman F, Heim S. Quantitative acute leukemia cytogenetics. *Genes Chrom Cancer*. 1992;5:57–66.
5. Mitterbauer M, Laezika K, Novak M, et al. High concordance of karyotype analysis and RT-PCR for CBFb/MYH11 in unselected patients with acute myeloid leukemia. A single center study. *Am J Clin Pathol*. 2000;113:406–410.
6. Razzouk BI, Raimondi SC, Srivastava DK, et al. Impact of treatment on the outcome of acute myeloid leukemia with inversion 16: a single institution's experience. *Leukemia*. 2001;15:1326–1330.
7. Poirel H, Radford-Weiss I, Rack K, et al. Detection of the chromosome 16 CBFb-MYH11 fusion transcript in myelomonocytic leukemias. *Blood*. 1995;85:1313–1322.
8. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:620–624.
9. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:81–87.
10. Arber DA, Brunning RD, Le Brunning RD, et al. Acute myeloid leukemia with inv(16)(p13.1;q22) or t(16;16)(p13.1;q22); CBFb-MYH11. In: Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:111–112.
11. Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
12. Moscinski LC, Kasnic G Jr, Saker A Jr, et al. The significance of an elevated serum lysozyme value in acute myelogenous leukemia with eosinophilia. *Am J Clin Pathol*. 1992;97:195–201.
13. Nakamura H, Sadamori N, Tagawa M, et al. Inversion of chromosome 16 in bone marrow eosinophils of acute myelomonocytic leukemia (M4) with eosinophilia. *Cancer Genet Cytogenet*. 1987;29:327–330.

14. Abbondanzo SL, Gray RG, Whang-Pang J, et al. A myelodysplastic syndrome with marrow eosinophilia terminating in acute nonlymphocytic leukemia, associated with an abnormal chromosome 16. *Arch Pathol Lab Med*. 1987;111:330–332.
15. Brown NJ, Stein RS. Idiopathic hypereosinophilic syndrome progressing to acute myelomonocytic leukemia. *South Med J*. 1989;82:1303–1305.
16. Sun X, Medeiros LJ, Lu D, et al. Dysplasia and high proliferation rate are common in acute myeloid leukemia with inv(16)(p13q22). *Am J Clin Pathol*. 2003;120:236–245.
17. Adriaansen HJ, te Broekhorst PAW, Hagemeijer AM, et al. Acute myeloid leukemia M4 with bone marrow eosinophilia (M4Eo) and inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression. *Blood*. 1993;81:3043–3051.
18. Hans CP, Finn WG, Singleton TP, et al. Usefulness of anti-CD117 in the flow cytometric analysis of acute leukemia. *Am J Clin Pathol*. 2002;117:301–305.
19. Paietta E, Papenhausen P, Azar C, et al. Inv(16) occurring in a case of acute biphenotypic leukemia lacking monocytic markers: multiple but short remissions. *Cancer Genet Cytogenet*. 1987;25:367–368.
20. Ferrara F, Vecchio LD. Acute myeloid leukemia with t(8;21)/AML1/ETO: a distinct biological and clinical entity. *Haematologica*. 2002;87:306–319.
21. Castilla LH, Perrat P, Martinez NJ, et al. Identification of genes that synergize with Cbfb-MYH11 in the pathogenesis of acute myeloid leukemia. *Proc Natl Acad Sci U S A*. 2004;101:4924–4929.
22. Trnkova Z, Pekova S, Bedrlikova R, et al. Type J Cbfbeta/MYH11 transcript in the M4Eo subtype of acute myeloid leukemia. *Hematology*. 2003;8:115–117.
23. Schsnitinger S, Bacher U, Haderlach C, et al. Rare Cbfb-MYH11 fusion transcripts in AML with inv(16)/t(16;16) are associated with therapy-related AML M4Eo, atypical cytomorphology, atypical immunophenotype, atypical additional chromosomal rearrangements and low white blood cell count: a study on 162 patients. *Leukemia*. 2007;21:725–731.
24. Dierlamm J, Stul M, Vranckx H, et al. FISH identifies inv(16)(p13q22) masked by translocations in three cases of acute myeloid leukemia. *Genes Chrom Cancer*. 1998;22:87–94.
25. Hernandez JM, Gonzalez MB, Granada I, et al. Detection of inv(16) and t(16;16) by fluorescence in situ hybridization in acute myeloid leukemia M4Eo. *Haematologica*. 2002;85:481–485.
26. Egan N, O'Reilly J, Chipper L, et al. Deletion of Cbfb in a patient with acute myelomonocytic leukemia (AML M4Eo) and inversion 16. *Cancer Genet Cytogenet*. 2004;154:60–62.
27. Ravandi F, Kaskol SS, Ridgeway J, et al. Molecular identification of Cbfb-MYH11 fusion transcripts in an AML M4Eo patient in the absence of inv16 or other abnormality by cytogenetic and FISH analyses—a rare occurrence. *Leukemia*. 2003;17:1907–1910.
28. Grinwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood*. 1998;92:2322–2333.
29. Schoch C, Buchner T, Freund M, et al. Fifty-nine cases of acute leukemia with inversion inv(16)(p13q22): do additional chromosomal aberrations influence prognosis? In: Buchner T, et al., eds. *Acute Leukemias VI. Prognostic Factors and Treatment Strategies*. Berlin-Heidelberg: Springer-Verlag; 1997:11–16.
30. Mrozek K, Marcucci G, Paschka P, et al. Advances in molecular genetics and treatment of core-binding factor acute myeloid leukemia. *Curr Opin Oncol*. 2008;20:711–718.
31. Arber DA, Heerema-McKenney A. Acute myeloid leukemia. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:672–697.
32. Polard JA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic significance of KIT mutations in pediatric patients with core binding factor AML enrolled on serial pediatric cooperative trials for de novo AML. *Blood*. 2011;115:2372–2379.
33. Luck SC, Russ AC, Du J, et al. KIT mutations confer a distinct gene expression signature in core binding factor leukaemia. *Br J Haematol*. 2010;148:925–937.
34. Chan NPH, Wong WS, Ng MHL, et al. Childhood acute myeloid leukemia with Cbfb-MYH11 rearrangement: study of incidence, morphology, cytogenetics, and clinical outcomes of Chinese in Hong Kong. *Am J Hematol*. 2004;76:300–303.
35. Martinet D, Muhiematter D, Leeman M, et al. Detection of 16 p deletions by FISH in patients with inv(16) or t(16;16) and acute myeloid leukemia (AML). *Leukemia*. 1997;11:964–970.

CASE 7

Acute Promyelocytic Leukemia with t(15;17)

CASE HISTORY

A 53-year-old man was admitted to the hospital because of continuous nosebleeds and gum bleeding after tooth brushing over 5 days prior to admission. The patient was in his usual state of health until 2 weeks ago when he developed fatigue, night sweats, fevers, and chills after visiting his dying mother in another state. He also claimed to have lost 5 lb since then. However, it was his nosebleeds that brought him to the hospital.

His physical examination on admission showed no bruises or petechiae on the skin and no hepatosplenomegaly. His total leukocyte count was 1,900/ μ L, hematocrit 24%, hemoglobin 8.5 g/dL, and platelets 19,000/mL. His absolute neutrophil count was 100/mL, lymphocytes 1,600/mL, and monocytes 100/mL. Coagulation studies revealed fibrinogen 257 mg/dL, prothrombin time 15 seconds, and absence of D-dimer. Because of the presence of atypical bilobed cells in the peripheral blood, a bone marrow biopsy was performed (Fig. 6.7.1), which demonstrated features of acute promyelocytic leukemia (APL). Cytogenetic study of the bone marrow revealed t(15;17).

The patient was treated with all-trans-retinoic acid (ATRA) leading to complete remission. However, despite consolidation therapy, the patient had a relapse of leukemia 18 months after initial treatment. The patient has since switched to arsenic trioxide (ATO) treatment and is now in complete remission.

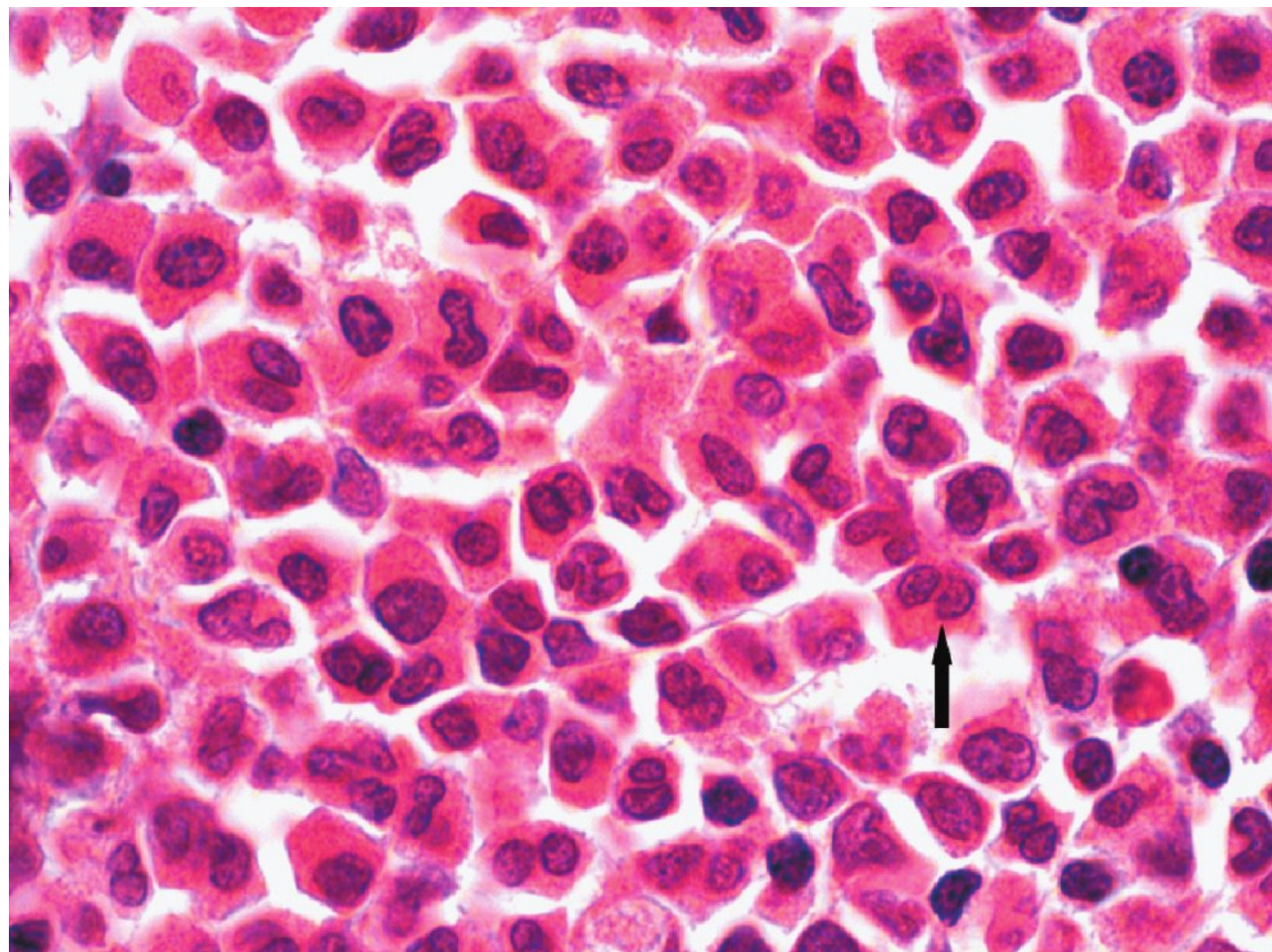


FIGURE 6.7.1 Bone marrow core biopsy shows total replacement of normal hematopoietic cells by the leukemic promyelocytes. Note many bilobed cells (arrow) are present. The abundant eosinophilic cytoplasm represents hypergranularity. Hematoxylin and eosin, 100 $\times$ magnification.

FLOW CYTOMETRY FINDINGS

Bone marrow: B-cell markers: CD19 0%, k 0%, l 0%; T-cell markers: CD3 0%, CD7 0%; myeloid markers: CD13-CD33 99%, myeloperoxidase (MPO) 75%, CD14 1%, HLA-DR 2%. Stem cell markers: CD34 52% (Fig. 6.7.2).

CYTOCHEMICAL FINDINGS

Leukemic cells from both the peripheral blood and bone marrow were positive for MPO and chloroacetate esterase (CAE) stains, but were negative for α -naphthyl butyrate esterase (NBE) stain. CAE stain also demonstrated single and multiple Auer rods in leukemic cells (Fig. 6.7.3).

DISCUSSION

APL accounts for only 5% to 13% of all cases of acute myeloid leukemia (AML), but it is the most well-defined subtype of AML (1). Cytologically, it differs from other AML in that the leukemic cells are not at the blastic stage but are atypical promyelocytes. Clinically, it is characterized by the presence of leukopenia in most cases rather than leukocytosis as seen in other leukemia and by the frequent existence of a hemorrhagic syndrome at the acute stage. Molecular genetically, it shows a nonrandom karyotype of t(15;17)(q22;q12) with the molecular characteristic of promyelocytic leukemia (PML)/retinoic acid receptor α (RAR α) fusion transcript in the majority of cases. It is one of the rare examples of leukemia for which an effective treatment has been established by understanding its molecular genetic abnormality. The therapeutic mechanism of ATRA is to induce differentiation of the leukemic cells by reversing the transcriptional repression of PML-RAR α .

Morphology and Cytochemistry

The leukemic cells in most cases of APL assume the morphology of hypergranular promyelocytes and are considered the typical cells in AML-M3 (French-American-British [FAB] classification). These leukemic cells are generally larger (14 to 25 nm) than normal promyelocytes and are devoid of a prominent paranuclear clear Golgi region, as is frequently seen in normal promyelocytes (2). The most characteristic feature is the abundance of cytoplasmic granules that cover the entire cytoplasm and mask the nucleus of the leukemic cells (Figs. 6.7.4–6.7.6). The nuclei of the APL cells show a great variation both in size and in shape, but many of them are kidney-shaped or bilobed. The cytoplasmic granules are believed to contain MPO, procoagulant substances, and bactericidal enzymes (3).

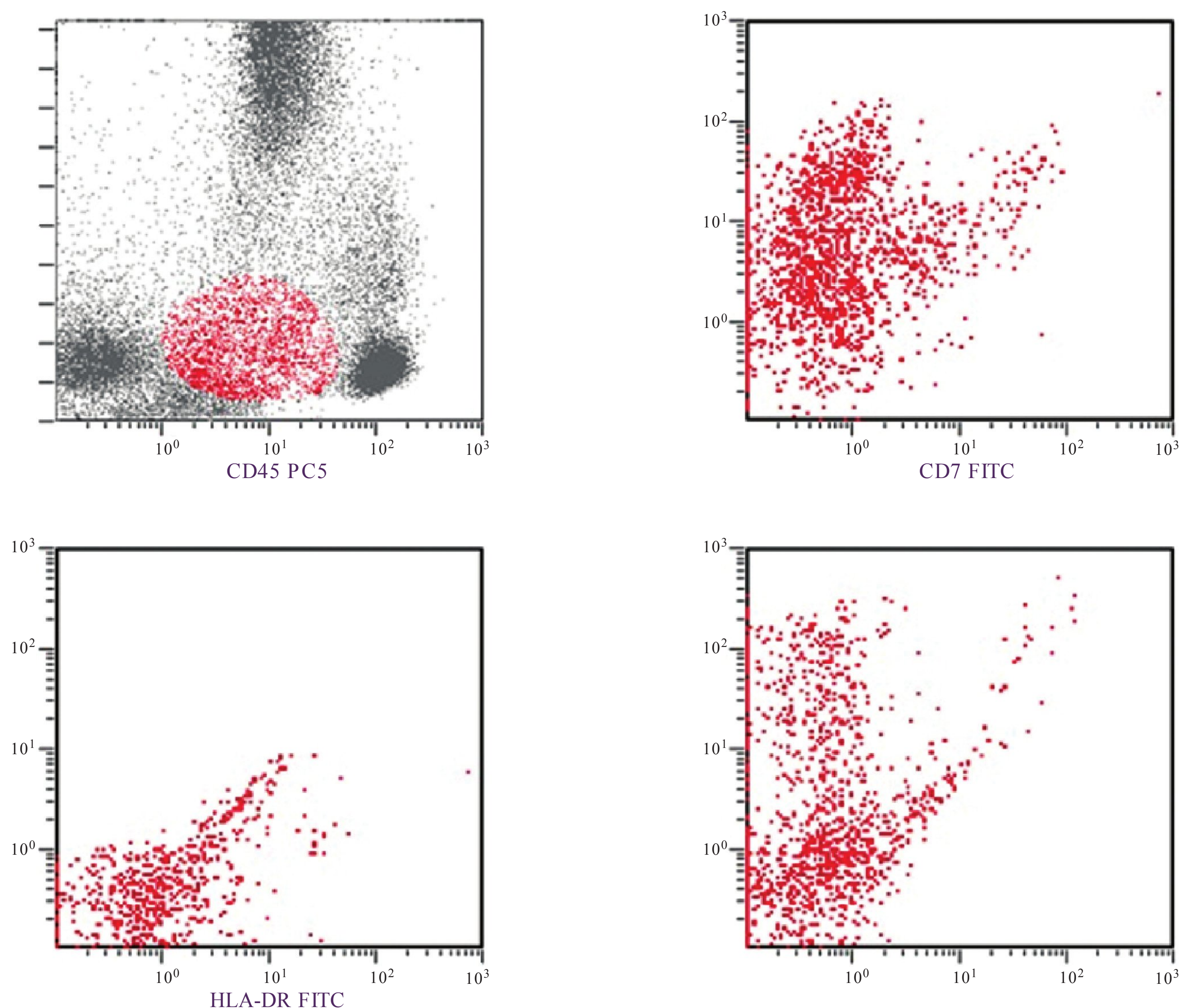


FIGURE 6.7.2 Flow cytometric histograms show strongly positive CD33 and weakly positive CD34 but negative HLA-DR. SS, side scatter; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine; PC5, phycoerythrin–cyanin 5.

However, the above features are not diagnostic for APL unless multiple Auer rods in bundles are demonstrated in the cytoplasm of the leukemic cells. These cells are commonly referred to as faggot cells. The Auer rods in APL cells show an internal, hexagonal tubular structure with a periodicity of 22 to 25 nm in contrast to the 8 to 12 nm periodicity of the Auer rods observed in other types of AML (4).

In approximately 15% to 20% of APL cases, the leukemic cell contains only a few cytoplasmic granules or the granules are so small (<250 nm resolution of light microscopy) that they can only be demonstrated by electron microscopy (5–7). These cases are coined hypogranular or microgranular APL, respectively, and are designated AML-M3v in the FAB classification. In M3v cases, the nuclei of the leukemic cells are usually folded or bilobed, mimicking those of monocytes (Fig. 6.7.7).

A rare type of hyperbasophilic microgranular variant is characterized by cells with a high nuclear cytoplasmic

ratio, strongly basophilic cytoplasm with sparse or no granules, and conspicuous cytoplasmic budding mimicking micromegakaryocytes (8,9). A hand-mirror variant of M3v has also been described (10).

APL cases usually are positive for both MPO and CAE stains. MPO and Sudan black B stains are usually strongly positive (4,9). CAE is particularly helpful in demonstrating the multiple Auer rods. MPO may also serve the same function, but false-positive results may occur due to precipitation of reagents. Among nonspecific esterase, NBE is generally negative in APL cases, but NBE reaction can be seen in certain subgroups of APL cases (11).

Immunophenotype

Because APL may mimic acute monocytic leukemia, flow cytometry is particularly helpful in demonstrating positive myelomonocytic antigens (CD13, CD15, and CD33) but negative monocytic antigens (CD14 including My4, Leu M3,

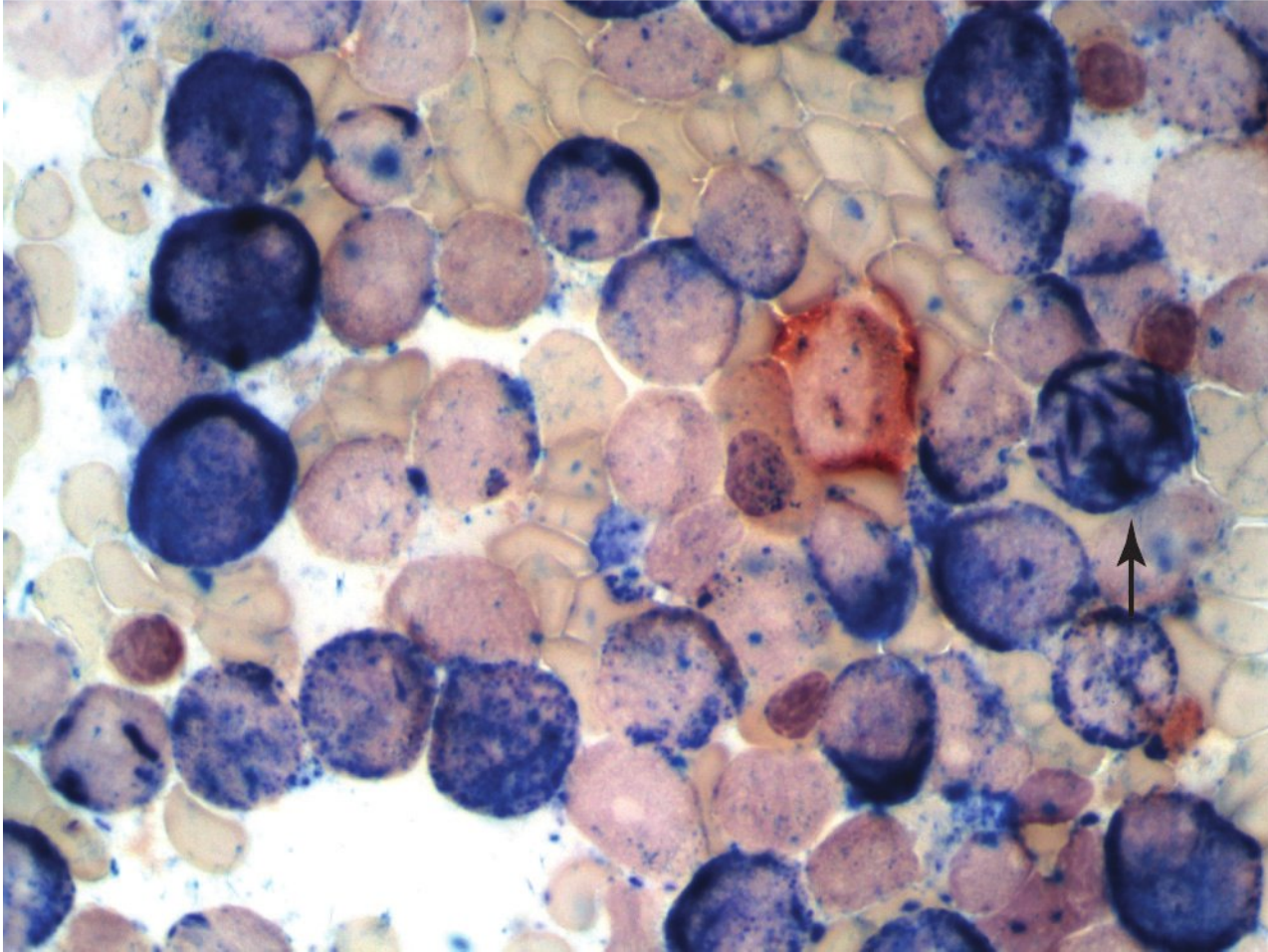


FIGURE 6.7.3 Combined esterase stain of the bone marrow aspirate shows multiple Auer rods demonstrated by CAE (blue) stain in a few leukemic cells (arrow). 100× magnification.

and Mo2) except for CD64 (3,12–18). However, the most important diagnostic feature is the absence or low percentage of HLA-DR. HLA-DR is present in myeloblasts and monoblasts but not promyelocytes; therefore, the absence of HLA-DR distinguishes APL from other subtypes of AML. However, positive conversion of HLA-DR can be present in APL cases at time of relapse (19). As HLA-DR is also absent in normal promyelocytes, APL has to be distinguished from reactive promyelocytosis. Postchemotherapy specimens from other AML subtypes sometimes show synchronous regeneration of promyelocytes that may also mimic APL (Fig. 6.7.8).

The stem cell marker, CD34, is frequently absent in APL cases and is also used to separate APL from other AML cases (13,17–20). However, CD34 positivity was demonstrated in 41% of APL cases in one study (21). Those

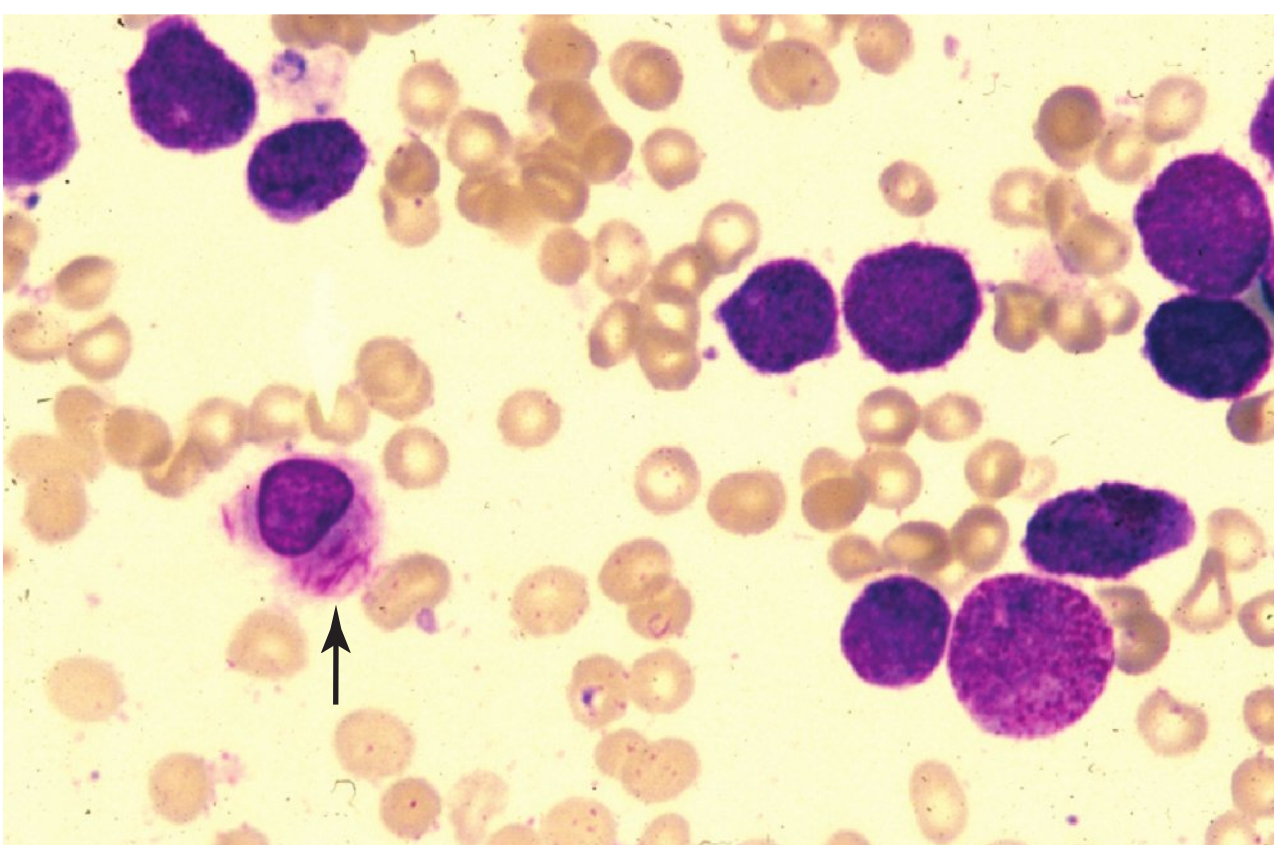


FIGURE 6.7.4 Peripheral blood smear shows several hypergranular promyelocytes with the nuclei being masked by the cytoplasmic granules. Note one promyelocyte contains multiple Auer rods (arrow). Wright–Giemsa, 100× magnification.

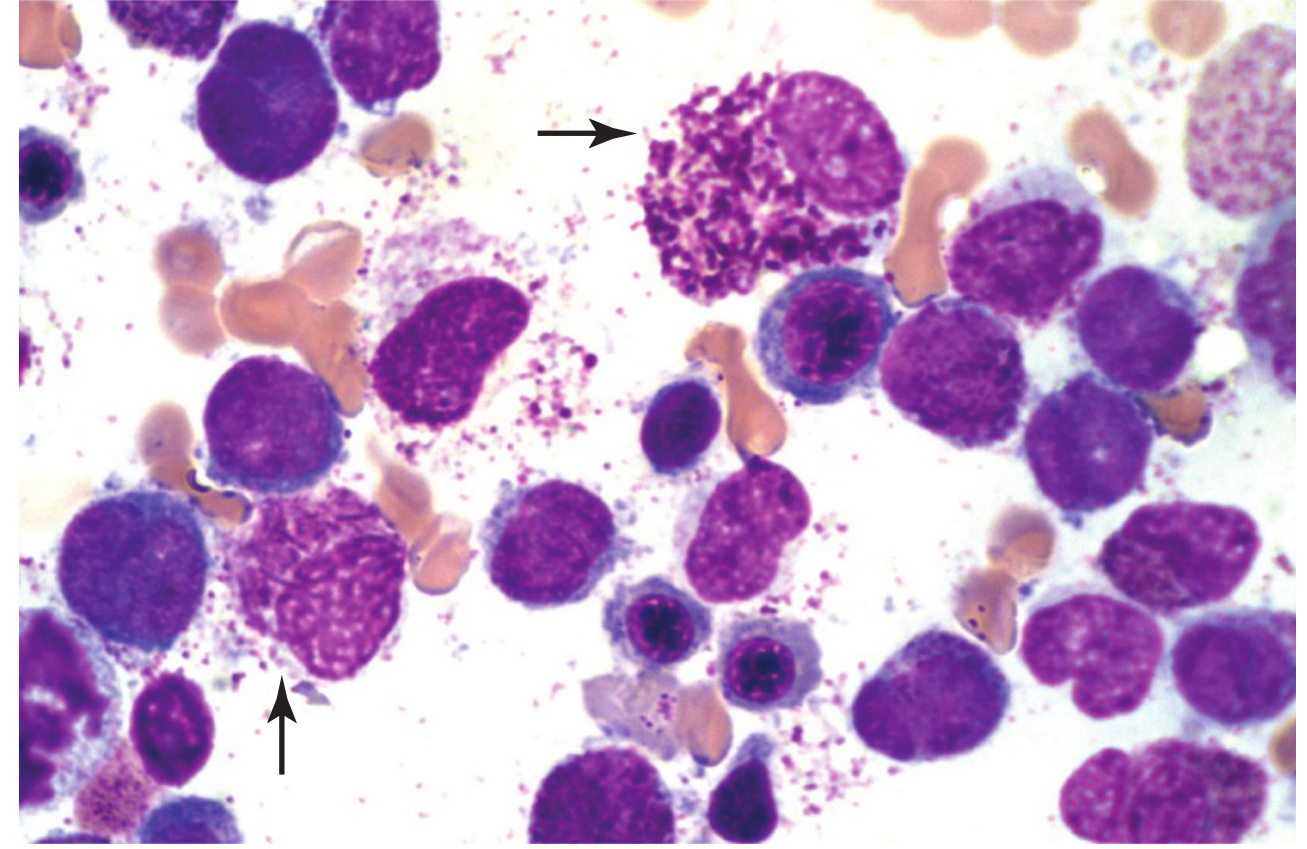


FIGURE 6.7.5 Bone marrow aspirate shows many leukemic promyelocytes, and two reveal multiple Auer rods (arrows). Wright–Giemsa, 100× magnification.

CD34-positive cells harbored the t(15;17) translocation as identified by the fluorescence in situ hybridization (FISH) technique. Another study claimed that the presence of a heterogeneous expression of CD13 and dual staining of CD34 and CD15 were highly characteristic of APL with a sensitivity of 100% and specificity of 99% for predicting PML-RARa gene rearrangement (22). In M3v cases and cases with bcr-3 transcript of the PML-RARa fusion gene, the expression of CD34 is common (23).

The addition of CD117 (c-kit) to the immunophenotypic panel is very helpful in distinguishing APL from reactive promyelocytosis (24). CD11b will further separate these two entities (24). In a recent study, 77% of APL cases were CD117+ CD11b–, whereas all cases recovered from agranulocytosis were CD117– CD11b+ (24). A panel composed of leukocyte integrin-associated antibodies, including CD11a,

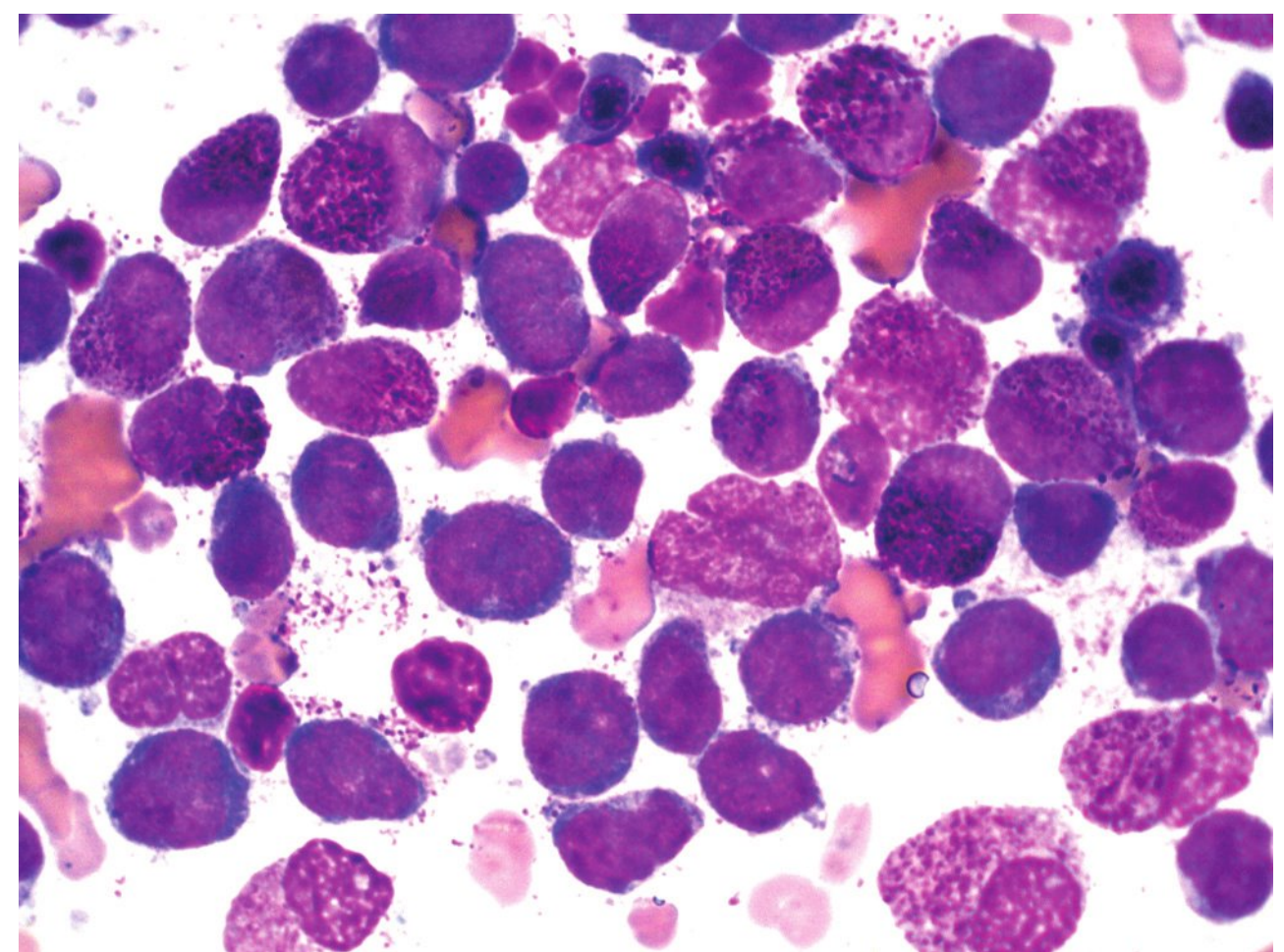


FIGURE 6.7.6 Bone marrow aspirate shows many hypergranular promyelocytes. Note that the cytoplasmic granules either push the nucleus to one side or mask the entire nucleus. Wright–Giemsa, 100× magnification.

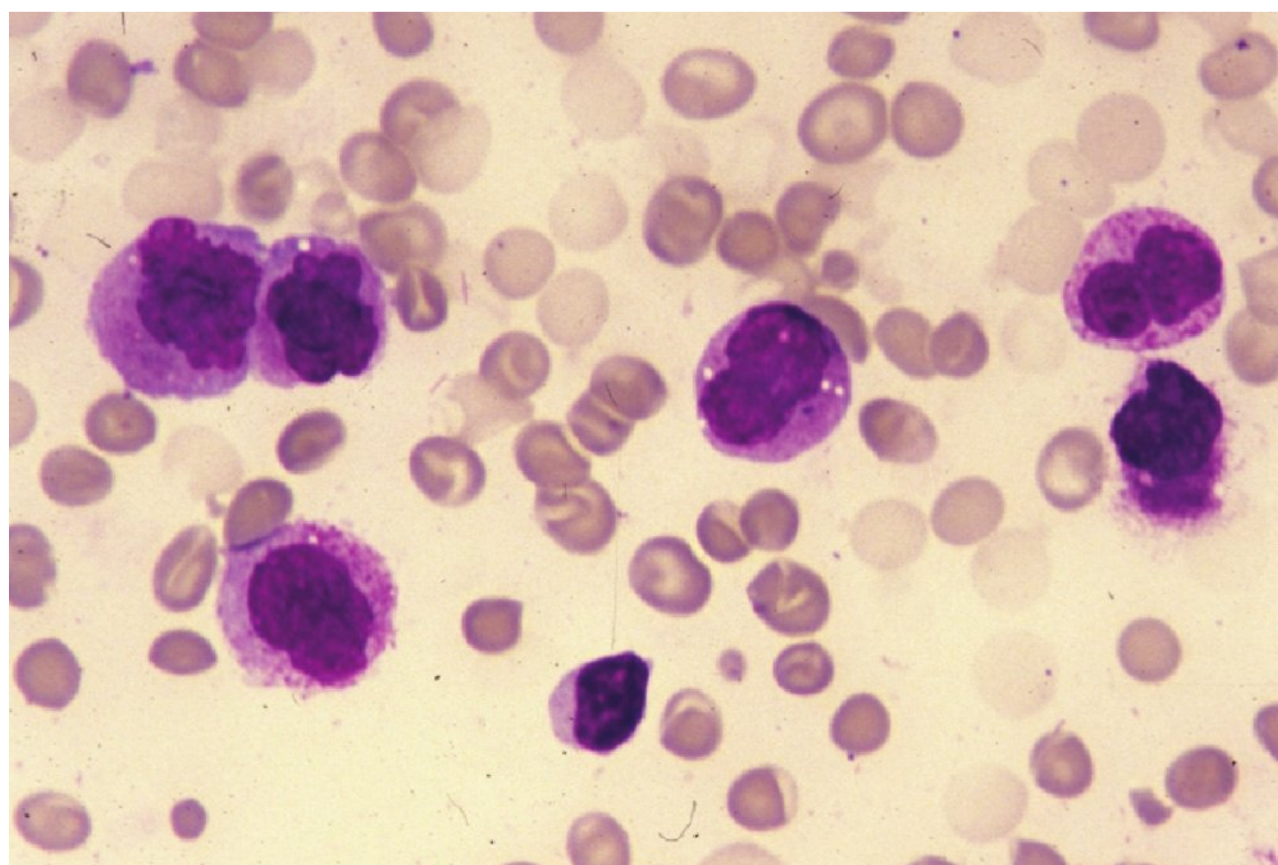


FIGURE 6.7.7 Peripheral blood smear shows several hypogranular promyelocytes with bilobed or folded nuclei, mimicking monocytes. Wright-Giemsa, 100× magnification.

CD11b, and CD18, is useful to distinguish between APL and other AML subtypes (25). PML-RARa-positive APL cells typically lack leukocyte integrins and show low percentages of the above markers.

Another useful marker is CD2 (a T-cell marker), which is frequently demonstrated in M3v but rarely in hypergranular M3 (12,13). A recent study showed that expression of CD2 in M3 correlates with the short type of PML-RARa transcript and with poor prognosis (26). CD7, another T-cell marker, may be demonstrated in other subtypes of AML, but is consistently absent in APL (27,28).

Antibodies to the PML gene product are now available for immunohistochemical and immunofluorescent stains (29–31). PML protein is present in the nucleus of normal cells and is characterized by a speckled pattern, which is the presence of 5 to 20 nuclear particles (nuclear bodies) per nucleus. The APL cells, in contrast, show a micro-speckled or microgranular pattern, which is composed of >50 granules. This phenomenon is the result of disruption

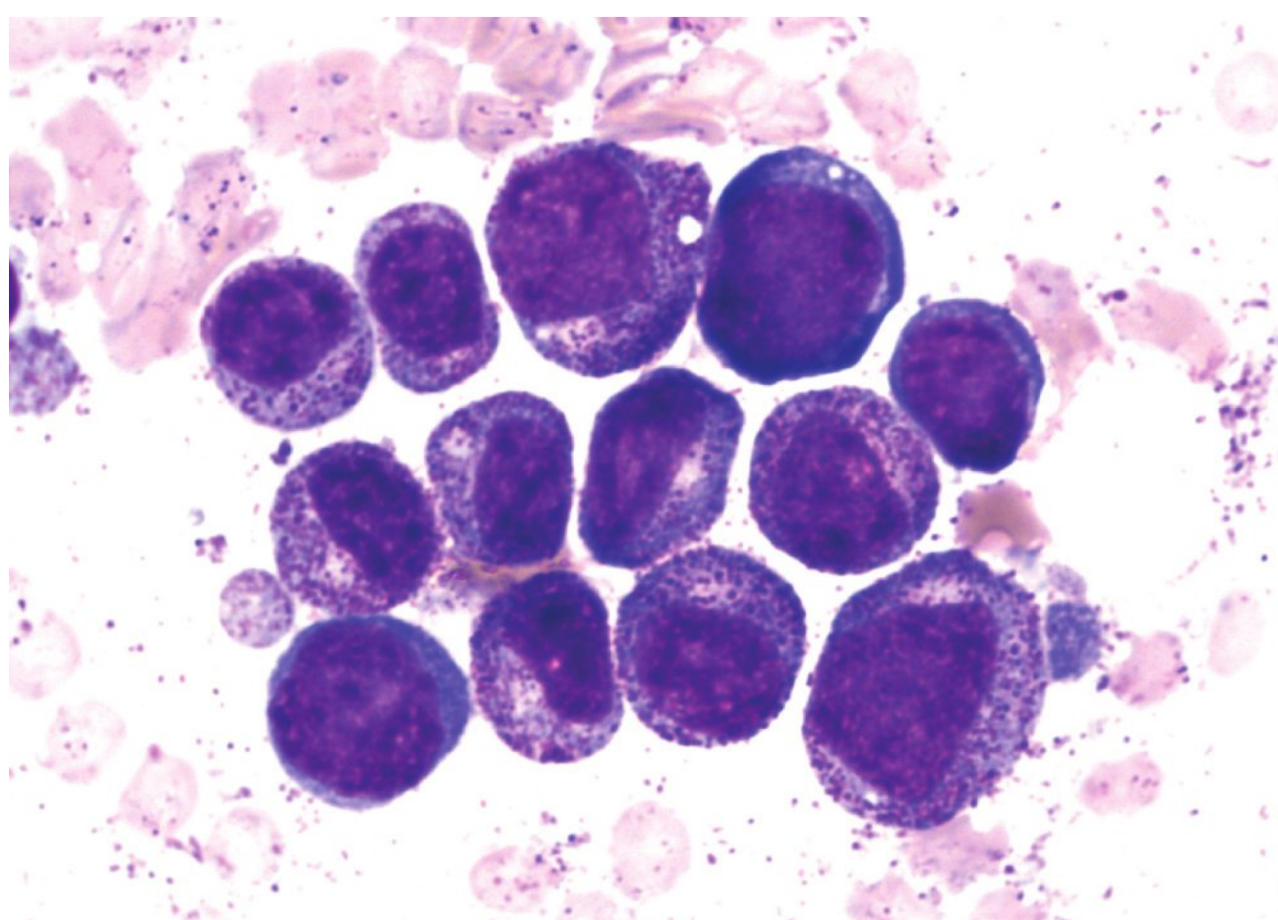


FIGURE 6.7.8 Bone marrow aspirate shows a cluster of hypergranular promyelocytes due to synchronous proliferation of promyelocytes after chemotherapy of M2 leukemia. Wright-Giemsa, 100× magnification.

of the nuclear bodies and redistribution of the protein in the APL cells. It is a reliable and simple technique and can be used for therapeutic monitoring. After treatment, the PML nuclear pattern may return to being speckled. A recent immunofluorescence study of 349 APL patients in the MD Anderson Cancer Center showed that immunostaining with anti-PML antibody is highly sensitive (98.9%) and specific (98.7%) and is far superior to the conventional cytogenetics (32). Because of its short turnaround time of 4 hours, this will prove to be a very efficient screening tool.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry can be used to make the initial diagnosis of APL and for therapeutic monitoring. CD2 may help to predict the prognosis. Immunohistochemical staining for PML protein is considered a reliable technique, but it is not yet commonly used in histology laboratories.

Molecular Genetics

Approximately 99% of APL cases including M3 and M3v show t(15;17)(q22;q21) translocation (Fig. 6.7.9), which produces a PML-RARa or, to a lesser extent, RARa-PML fusion transcript (3,20,33–35). The remaining APL cases involve three partner genes translocating with RARa: PML zinc finger (PLZF) (also known as ZBTB16) in t(11;17)(q23;q21), nucleophosmin (NPM) gene in t(5;17)(q23;q12), nuclear matrix-associated gene (NuMA) in t(11;17)(q13;q21), and STAT5B gene in t(17;17)(q11;q21) (1,16,29,35,36) (Table 6.7.1). Leukemic cells with the PLZF/RARa or STAT5B/RARa fusion products are resistant to ATRA therapy. PLZF/RARa subgroup also shows distinct morphologic features, such as lack of bilobed or folded nuclei, rarity of Auer rods and faggot cells, and an increased number of Pelgeroid neutrophils (37).

In addition, reverse transcriptase-polymerase chain reaction (RT-PCR) demonstrates three isoforms in PML-RARa transcripts: L (long) type, V (variable) type, and S (short) type containing bcr-1, bcr-2, and bcr-3, respectively (29). The bcr-3 transcript is associated with higher white blood cell counts, M3v morphology, additional karyotypic abnormalities, and the expression of CD34 and CD2 (29). Interestingly, Latin American patients have a high frequency of bcr-1 subtype, which was also demonstrated in a small cohort of Chinese APL patients and in a Japanese group (38). It was proposed that this phenomenon might be related to a non-European genetic factor. Additional cytogenetic aberrations, most frequently involving chromosomes 3, 8, 7, and 11, are more often seen in relapsed APL cases (19).

RARa regulates transcription of ATRA target genes and recruits the nuclear corepressor (N-CoR)/histone-deacetylase (HD) complex, which lead to a repressive chromatin conformation (29). As a result, there is a developmental arrest at the promyelocytic stage. High doses of ATRA release HD activity from PML-RARa but not from PLZF-RARa, because the latter contains a second N-CoR/HD binding site in the PLZF moiety (29). This explains why ATRA is not effective in treating cases with PLZF-RARa.

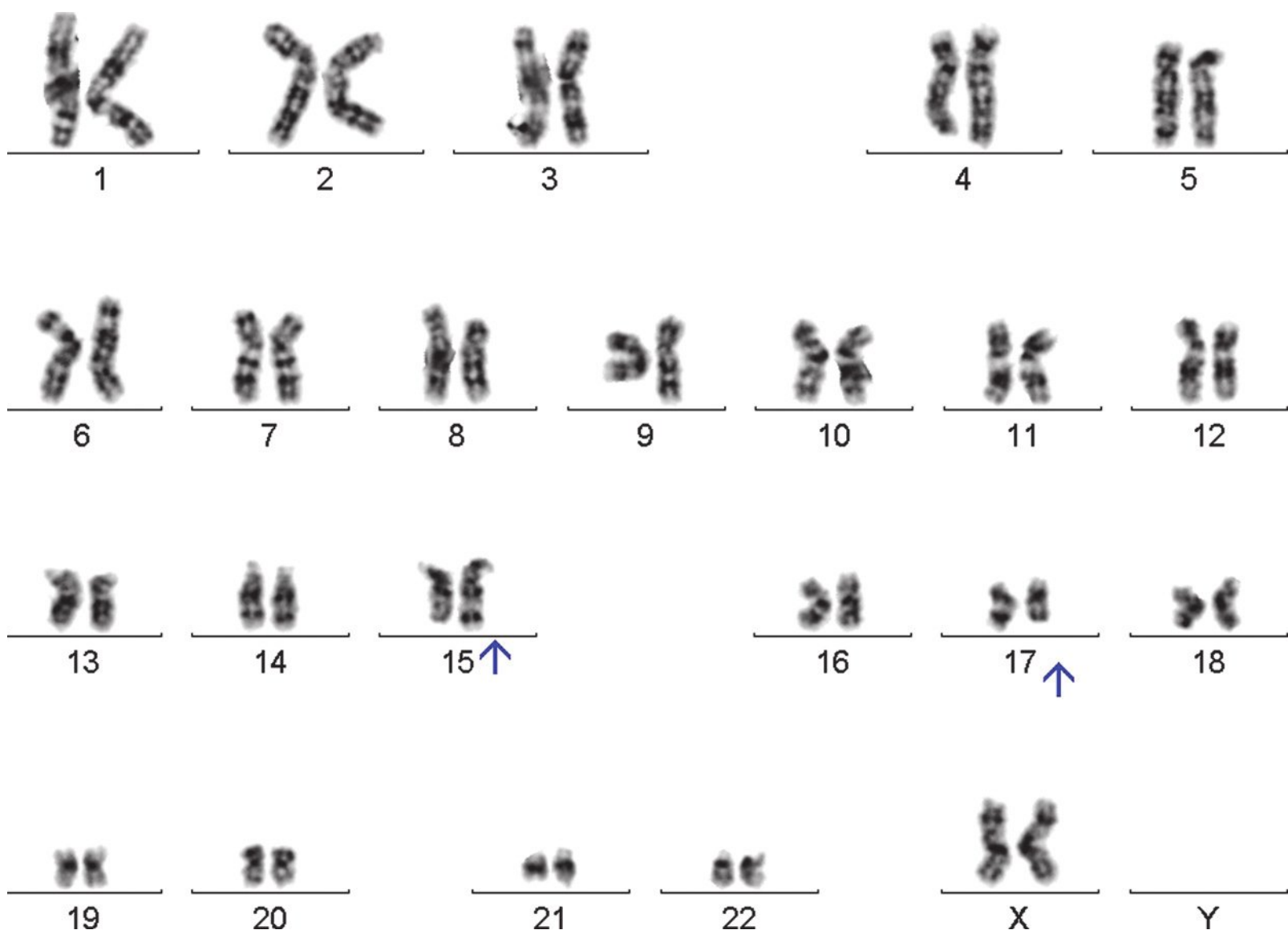


FIGURE 6.7.9 Cytogenetic karyotype shows t(15;17)(q22;q21).

The mechanism for ATRA resistance in patients with STAT5B-RAR α is different. In those cases, it is probable that the coiled-coil domain of STAT5 is responsible for impaired differentiation in hematopoietic cells and ATRA-insensitive interaction with a corepressor complex, contributing to the development of an ATRA-resistant form of APL (39).

The action of ATRA is to induce the leukemic promyelocyte to differentiate terminally. However, ATRA alone may not be sufficient; therefore, the current protocol is the combination of ATRA and anthracycline-containing chemotherapy (16,29). After treatment, the bone marrow is replenished with terminally differentiated granulocytes, in contrast to the hypocellular bone marrow seen in other leukemia immediately after chemotherapy (33). At the molecular level, the ATRA effect is mediated by the

transcription factor FOXO3A in APL cells (40). FOXO3A is phosphorylated and becomes inactivated in APL cells, but ATRA dephosphorylates FOXO3A and activate its target genes, such as the tumor necrosis factor-related apoptotic-inducing ligand, so that FOXO3A returns to normal function (40).

Recently, ATO has been used to treat APL patients resistant to ATRA. ATO may be similar to ATRA in inducing APL cell differentiation through disruption of the PML-RAR α function (41,42). In addition, ATO also induces apoptosis of leukemic cells (42).

A study of gene expression profiling identified two major clusters in APL cases, corresponding to the two morphologic subtypes (7). The first cluster was represented by cases with M3v morphology, high leukocyte count, bcr-3 PML-RAR α isoform, and Flt3-ITDs (Fms-like tyrosine kinase

TABLE 6.7.1					
Comparison of Various Molecular Genetic Forms in APL					
	PML-RAR α	PLZF-RAR α	NPM-RAR α	NuMA-RAR α	STAT5b-RAR α
Karyotypes	t(15;17)(q22;q21)	t(11;17)(q23;q21)	t(5;17)(q23;q12)	t(11;17)(q13;q21)	t(17;17)(q11;q21)
Breakpoint variants	3 breakpoints	Most cases include first 2 Zn fingers	2 Fusion cDNAs alternative splicing	1 Breakpoint	1 Breakpoint—internal deletion of chromosome 17
Nuclear body pattern	Microspeckles or cytoplasmic	Microspeckles, not cytoplasmic	Microspeckles	Sheet-like aggregates	Microspeckles
ATRA response	Sensitive	Resistant	Sensitive	Sensitive	Resistant
Reciprocal translocation	Present in 70–80% of cases	Present in all cases tested	Identified in index case	Not reported	Not present

APL, acute promyelocytic leukemia; ATRA, all-trans-retinoic acid; NPM, nucleophosmin; NuMA, nuclear matrix-associated; PLZF, promyelocytic leukemia zinc finger; PML, promyelocytic leukemia; RAR α , retinoic acid receptor α

3–internal tandem duplications). The second cluster was composed of cases with typical M3 morphology, bcr-1 PML-RARa isoform, leucopenia, and Flt3-WT (Flt3-wild-type).

As PML-RARa transgenic mice only develop a nonfatal myeloproliferative disorder, additional mutations are probably required to produce overt leukemia. A candidate gene to play such a role is Flt3 (7). For instance, Flt3-ITDs up-regulate the hyperleukocytosis gene and blood coagulation gene clusters. It also down-regulates genes that encode for proteins present in granulocytic granules leading to the hypogranular variant form (7). In treatment-related APL, Flt3 mutation and secondary cytogenetic abnormalities are more frequently detected than de novo APL (43). A systematic review of 11 studies showed that the incidence of Flt3-ITD mutation was 12% to 38% and Flt3-tyrosine kinase domain (TKD) mutation, 2% to 20% (44). Both mutations were associated with adverse survival for patients (44).

Whereas molecular genetic confirmation is mandatory in the diagnosis of APL, treatment should be started as soon as possible even before genetic evidence is available, because the patient may die in the early stage of the disease due to acute hemorrhage. Molecular genetic techniques are useful not only for the diagnosis but also for follow-up of the patients for minimal residual disease (MRD). However, molecular evaluation is reliable only at the end of consolidation and not immediately after induction therapy (31).

Conventional karyotyping may help to diagnose 80% to 90% of APL cases. Its major advantage is its capability to detect additional chromosomal abnormalities besides t(15;17) and to identify other APL variants (29). However, it is a time-consuming procedure, a good-quality bone marrow metaphase is not always obtainable for karyotyping, and it is an insensitive technique for the detection of MRD (20,31).

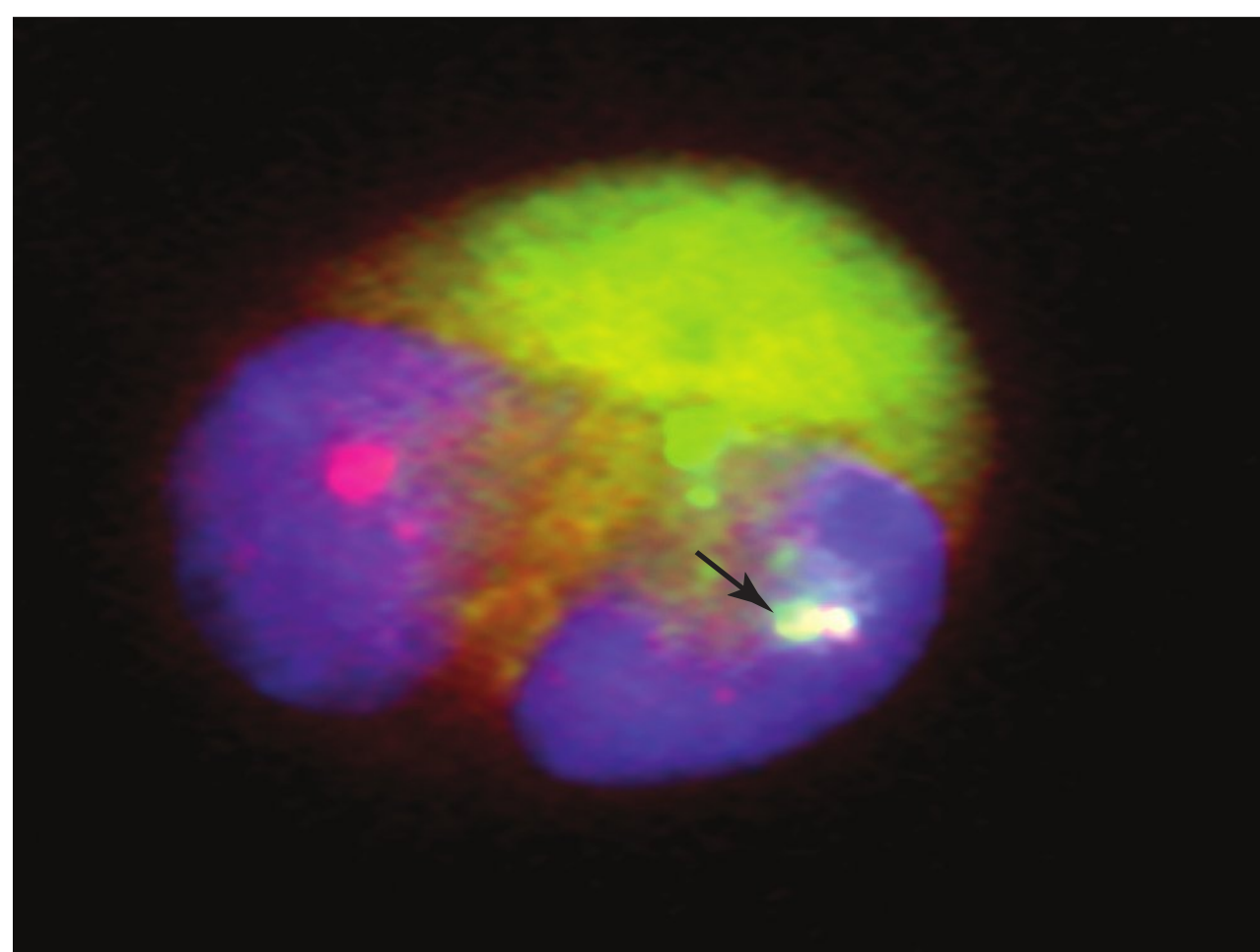


FIGURE 6.7.10 Fluorescence in situ hybridization of bone marrow aspirate with PML-RARa probes demonstrated one orange signal, one green signal, and the PML-RARa fusion product (arrow) in a leukemic promyelocyte with a bilobed nucleus. 100× original magnification.

FISH is a rapid and sensitive technique (29,31). It does not require dividing cells, so fresh specimens and culture technique are not needed. FISH is practically applicable to all kinds of specimens: blood smears, bone marrow, fresh tissues, and paraffin sections (Fig. 6.7.10). However, it cannot detect additional cytogenetic aberrations besides the targeted abnormality (i.e., t(15;17)).

Southern blot is highly specific, but it is time consuming and laborious (31,34). Additional probes are needed to detect different breakpoints or to rule out a variant translocation.

RT-PCR is the only technique that defines the PML breakpoint type and is suitable for monitoring MRD (16,29,31). After successful treatment, PML-RARa disappears from the leukocytes, and the reappearance of this fusion transcript predicts a relapse. This phenomenon is in marked contrast to t(9;22) in chronic myeloid leukemia and to t(8;21) in AML. In those cases, the transcript may persist for a long time and no relapse occurs (20). However, RT-PCR is prone to contamination and artifacts, and interlaboratory discordance has been reported (29,31). Therefore, real-time PCR is advocated to provide standardization (29).

In the current case, the diagnosis of APL is confirmed by karyotyping. Although the patient had symptoms of hemorrhages, his fibrinogen was normal and D-dimer was negative, so disseminated intravascular coagulation (DIC) was probably not present. The morphology of the leukemic cells is consistent with M3v. The cytochemical staining is most helpful in demonstrating the presence of multiple Auer rods, because treatment can be started with this finding and multiple Auer rods are not easily detected without special staining. The only atypical clinical feature in this case is leukopenia instead of leukocytosis that is commonly seen in M3v cases.

The salient features for laboratory diagnosis of APL are summarized in Table 6.7.2.

Clinical Manifestations

The early clinical presentation is leukopenia in typical M3 but marked leukocytosis in M3v, which may reach 200,000/mL. The leukocyte count is an important predictor for the prognosis (7,45). In a study of 239 cases, patients with a leukocyte count <10,000/mL had a higher complete remission rate (85% vs. 62%), reduced relapse risk (13% vs. 35%), and superior survival (80% vs. 57%) than those with a leukocyte count >10,000/mL.

Morbidity and mortality are mainly related to coagulopathy. Many patients die of early fatal hemorrhage, especially intracranial or intrapulmonary hemorrhage. The incidence of early hemorrhage varies from 8% to 47% (33), and the mortality rate due to hemorrhages is still as high as 10% even in patients receiving modern treatment (29). Malignant promyelocytes release procoagulant substances that activate the coagulation cascade and generate thrombin, and deplete fibrinogen, clotting factors, and platelets, so that patients with APL may have DIC, fibrinolysis, and proteolysis (33,46). Clinically, the resolution of coagulopathy is the first sign of response

TABLE 6.7.2

Salient Features for Laboratory Diagnosis of APL

- 1. Presence of >20% hypergranular (or microgranular) promyelocytes in the bone marrow
- 2. Presence of multiple Auer rods in the cytoplasm of leukemic cells
- 3. Cytochemical staining: strongly positive for MPO and CAE, but negative for α-naphthyl butyrate esterase
- 4. General immunophenotype: positive for myelomonocytic antigens (CD13, CD15, CD33) but negative for most monocytic antigens (CD14, including My4, Leu M3, and Mo2), except for CD64
- 5. Specific immunophenotype: low level or absence of HLA-DR, low level or absence of integrin-associated antibodies (CD11a/CD11b/CD11c/CD18), negative CD34 in most cases but positive CD117
- 6. Abnormal karyotype: t(15;17) and other variants detected by cytogenetic technique
- 7. Identification of PML-RARα and other variants by RT-PCR or FISH
- 8. PML antibody staining for abnormal PML protein pattern by immunohistochemistry or immunofluorescence

FISH, fluorescence in situ hybridization; PML, promyelocytic leukemia; RARα, retinoic acid receptor α; RT-PCR, reverse transcriptase-polymerase chain reaction.

to ATRA (33). Because M3v has higher leukocyte counts and more severe coagulopathy than the typical APL, its prognosis is generally worse than that of the latter (14).

After ATRA treatment, approximately 50% of patients may develop the retinoic acid syndrome, which includes fluid retention, hectic fever, pulmonary infiltrates, and pleural effusions (47). This potentially fatal syndrome should be promptly treated with high-dose corticosteroids. ATO can also induce the same syndrome in about one third of patients (47).

REFERENCES

1. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumour of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:81–87.

2. Innes DJ Jr, Hess CE, Bertholf MF, et al. Promyelocyte morphology differentiation of acute promyelocytic leukemia from benign myeloid proliferations. *Am J Clin Pathol*. 1987;88:725–729.

3. Stone RM, Mayer RJ. The unique aspects of acute promyelocytic leukemia. *J Clin Oncol*. 1990;8:1913–1921.

4. Litz CE, Brunning RD. Acute myeloid leukemias. In: Knowles DM, ed. *Neoplastic Hematopathology*. Baltimore, MD: Williams & Wilkins; 1992:1315–1349.

5. Bennett JM, Catovsky D, Daniel MT, et al. A variant form of hypergranular promyelocytic leukemia (M3). *Br J Haematol*. 1980;44:169–170.

6. Golomb HM, Rowley JD, Vardiman JW, et al. “Microgranular” acute promyelocytic leukemia: a distinct clinical, ultrastructural and cytogenetic entity. *Blood*. 1980;55:253–259.

7. Marasca R, Maffei R, Zucchini P, et al. Gene expression profiling of acute promyelocytic leukaemia identifies two subtypes mainly associated with Flt3 mutational status. *Leukemia*. 2006;20:103–114.

8. McKenna RW, Parkin J, Bloomfield CD, et al. Acute promyelocytic leukemia. A study of 39 cases with identification of a hyperbasophilic microgranular variant. *Br J Haematol*. 1982;50:201–214.

9. Castoldi GL, Liso V, Specchia G, et al. Acute promyelocytic leukemia: morphological aspects. *Leukemia*. 1994;8:1441–1446.

10. Sun T, Weiss R. Hand-mirror variant of microgranular acute promyelocytic leukemia. *Leukemia*. 1991;5:266–269.

11. Davey FR, Davis RB, McCallum JM, et al. Morphologic and cytochemical characteristics of acute promyelocytic leukemia. *Am J Hematol*. 1989;30:221–227.

12. Neame PB, Soamboonsrup P, Browman GP, et al. Classifying acute leukemia by immunophenotyping. A combined FAB-immunologic classification of AML. *Blood*. 1986;68:1355–1362.

13. Second MIC Cooperative Study Group. Morphologic, immunologic and cytologic (MIC) working classification of the acute myeloid leukemias. *Br J Haematol*. 1988;68:487–494.

14. Rovelli A, Biondi A, Rajnodi AC, et al. Microgranular variant of acute promyelocytic leukemia in children. *J Clin Oncol*. 1992;10:1413–1418.

15. Traweek ST. Immunophenotypic analysis of acute leukemia. *Am J Clin Pathol*. 1993;99:504–512.

16. Fenauz P, Chomienne C, Degos L. Acute promyelocytic leukemia. Biology and treatment. *Semin Oncol*. 1997;24:92–102.

17. Dunphy CH. Comprehensive review of adult acute myelogenous leukemia. Cytomorphological, enzyme cytochemical, flow cytometric, immunophenotypic, and cytogenetic findings. *J Clin Lab Anal*. 1999;13:19–26.

18. Arber DA, Brunning RD, Le Beau MM, et al. Acute promyelocytic leukaemia with t(15;17)(q22;q12); PML-RARα. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008:112–114.

19. Dimov ND, Medeiros LJ, Ravandi F, et al. Acute promyelocytic leukemia at time of relapse commonly demonstrates cytogenetic evidence of clonal evolution and variability in blast immunophenotypic features. *Am J Clin Pathol*. 2010;133:484–490.

20. Grignani F, Fagioli M, Alcalay M, et al. Acute promyelocytic leukemia. From genetics to treatment. *Blood*. 1994;83:10–25.

21. Edwards RH, Wasik MA, Finan J, et al. Evidence for early hematopoietic progenitor cell involvement in acute promyelocytic leukemia. *Am J Clin Pathol*. 1999;112:819–827.

22. Orfao A, Chillon MC, Bortoluci AM, et al. The flow cytometric pattern of CD34, CD15, and CD13 expression in acute myeloblasts leukemia is highly characteristic of the presence of PML-RARα gene rearrangements. *Haematologica*. 1999;84:405–412.

23. Exner M, Thalhammer R, Kapiotis S, et al. The "typical" immunophenotype of acute promyelocytic leukemia (APL-M3): does it prove true for the M3-variant? *Cytometry*. 2000;42:106–109.
24. Rizzatti EG, Garcia AB, Pothan H, et al. Expression of CD117 and CD11b in bone marrow can differentiate acute promyelocytic leukemia from recovering myeloid proliferations. *Am J Clin Pathol*. 2002;118:31–37.
25. Paietta E, Goloubeva O, Neuberg D, et al. Eastern Cooperative Oncology Group. A surrogate marker profile for PML/RAR alpha expressing acute promyelocytic leukemia and the association of immunophenotypic markers with morphologic and molecular subtypes. *Cytometry B Clin Cytom*. 2004;59:1–9.
26. Lin P, Hao S, Medeiros LJ, et al. Expression of CD2 in acute promyelocytic leukemia correlates with short form of PML-RARa transcripts and poor prognosis. *Am J Clin Pathol*. 2004;121:402–407.
27. Kita K, Miwa H, Nakase K, et al. Clinical importance of CD7 expression in acute myelocytic leukemia. *Blood*. 1993;81:2399–2405.
28. Del Poeta G, Stasi R, Venditti A, et al. CD7 expression in acute myeloid leukemia. *Blood*. 1993;82:2929–2930.
29. Lo Coco F, Diverio D, Falini B, et al. Genetic diagnosis and molecular monitoring in the management of acute promyelocytic leukemia. *Blood*. 1999;94:417–428.
30. Falini B, Flenghi L, Fagioli M, et al. Immunocytochemical diagnosis of acute promyelocytic leukemia (M3) with the monoclonal antibody PG-M3 (anti-PML). *Blood*. 1997;90:4046–4053.
31. Sanz MA, Tallman MS, Lo Coco F. Tricks of the trade for the appropriate management of newly diagnosed acute promyelocytic leukemia. *Blood*. 2005;105:3019–3025.
32. Dimov ND, Medeiros LJ, Kantarjian HM, et al. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody. *Cancer*. 2010;116:369–376.
33. Warrell RP Jr, de Thé H, Wang ZY, et al. Acute promyelocytic leukemia. *N Engl J Med*. 1993;329:177–189.
34. Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
35. Powell BL. Acute progranulocytic leukemia. *Curr Opin Oncol*. 2001;13:8–13.
36. Zelent A, Guidez F, Melnick A, et al. Translocations of the RARa gene in acute promyelocytic leukemia. *Oncogene*. 2001;20:7186–7203.
37. Sainty D, Liso V, Cantu-Rajnoldi A, et al. A new morphologic classification system for acute promyelocytic leukemia distinguishes cases with underlying PLZF/RARa gene rearrangements. *Blood*. 2000;96:1287–1296.
38. Douer D, Santillana S, Ramezani L, et al. Acute promyelocytic leukaemia in patients originating in Latin America is associated with an increased frequency of the bcr1 subtype of the PML/RARa fusion gene. *Br J Haematol*. 2003;122:563–570.
39. Mauer AB, Wichmann C, Gross A, et al. The Stat5-RARa fusion protein represses transcription and differentiation through interaction with a corepressor complex. *Blood*. 2002;99:2647–2652.
40. Sakoe Y, Sakoe K, Kirito K, et al. FOXO3A as a key molecule for all-trans retinoic acid-induced granulocytic differentiation and apoptosis in acute promyelocytic leukemia. *Blood*. 2010;115:3787–3795.
41. Chou WC, Dang CV. Acute promyelocytic leukemia: recent advances in therapy and molecular basis of response to arsenic therapies. *Curr Opin Hematol*. 2005;12:1–6.
42. Wang ZY, Chen Z. Acute promyelocytic leukemia: from highly fatal to highly curable. *Blood*. 2008;111:2505–2515.
43. Yin CC, Glassman AB, Lin P, et al. Morphologic, cytogenetic, and molecular abnormalities in therapy-related acute promyelocytic leukemia. *Am J Clin Pathol*. 2005;123:840–848.
44. Beitinjaneh A, Jang S, Roukoz, et al. Prognostic significance of FLT3 internal tandem duplication and tyrosine kinase domain mutations in acute promyelocytic leukemia: a systematic review. *Leuk Res*. 2010;34:831–836.
45. Burnett AK, Grimwade D, Solomon E, et al. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the randomized MRC Trial. *Blood*. 1999;93:4131–4143.
46. Tallman MS. The thrombophilic state in acute promyelocytic leukemia. *Semin Thromb Hemost*. 1999;25:209–215.
47. Camacho LH, Soignet SL, Chanel S, et al. Leukocytosis and the retinoic acid syndrome in patients with acute promyelocytic leukemia treated with arsenic trioxide. *J Clin Oncol*. 2000;18:2620–2625.

CASE 8

Acute Myeloblastic Leukemia without Maturation (General Introduction of Acute Myeloid Leukemia)

CASE HISTORY

A 62-year-old man presented with symptoms of unstable angina. He was scheduled to have cardiac catheterization, but the procedure was postponed due to the development of fever of unknown origin for 2 weeks. The fever workup included blood cultures, urine cultures, and computed tomography scan of the chest and maxilla; all examinations were negative and failed to show any evidence of infection.

Physical examination revealed no hepatosplenomegaly and no lymphadenopathy. The initial peripheral blood examination demonstrated pancytopenia with blasts and several nucleated red blood cells. Further examination showed a total leukocyte count of 24,100/mL with 61% blasts, 11% neutrophils, 24% lymphocytes, and 1% monocytes. The hematocrit was 27.3%, hemoglobin 9.5 g/dL, and platelets 33,500/ μ L.

A bone marrow biopsy was performed. A 500-cell count showed 85% myeloblasts, 6.4% monoblasts, 1.2% promyelocytes, 0.4% myelocytes, 0.8% metamyelocytes, 1.2% bands, and 1.4% segmented neutrophils. Megakaryocytes were decreased. The core biopsy revealed 80% cellularity with the presence of large sheets of immature myeloid cells. However, small clusters of erythroid cells and mature granulocytes were still visible.

After admission, the patient continued to have cyclical fevers and was started with cefepime. The patient was informed of the diagnosis of acute myeloid leukemia (AML), the prognosis, and treatment of the disease. He decided to forgo chemotherapy and seek possible palliative care at home. The patient was discharged with the instruction to follow up by visiting hematology/oncology and cardiology clinics.

FLOW CYTOMETRY FINDINGS

Bone marrow: Myeloperoxidase (MPO) 4%, CD13-CD33 86%, CD13-CD33/CD7 60%, HLA-DR 86%, CD14 0%, CD34 91%, CD117 47% (Fig. 6.8.1).

CYTOCHEMICAL FINDINGS

MPO and chloroacetate esterase (CAE) stains were positive, whereas a-naphthyl butyrate esterase (NBE) was negative in the bone marrow specimen.

DISCUSSION

The first comprehensive classification scheme for AML was proposed by the French-American-British (FAB) group, which was based on the combination of morphology and cytochemistry (1,2). Subsequently, immunophenotypic and molecular genetic criteria were included for substantiation of the diagnosis (3). However, the blast count is still the basis for the diagnosis of AML and for the distinction of AML from myelodysplastic syndrome and myeloproliferative neoplasms. For morphologic identification of blasts and other myeloid cells, the reader is referred to two review articles (4,5). The most interesting morphologic finding of AML in recent years is the myeloblasts with cup-like nuclei (6,7) (Fig. 6.8.2). When 10% or more of this blast population is identified in an AML case, it is frequently associated with NPM1 mutation, FLT3-internal tandem duplications (ITDs), and a distinctive immunophenotype (CD34-, HLA-DR-). We also found that the cup-like nucleus can trap the two probes used in fluorescence in situ hybridization, thus forming a false-positive fusion product (Fig. 6.8.3).

The basic requirement for the diagnosis of AML in the FAB system is that >30% of all nucleated marrow cells are blasts and <50% are erythroid precursors, except for erythroleukemia (3). On rare occasions, bone marrow may show <30% blasts, but >30% blasts are present in the peripheral blood. This condition has been accepted as AML by a National Cancer Institute-sponsored workshop (8). The FAB classification includes several subtypes of AML: acute myeloblastic leukemia without maturation (M1), acute myeloblastic leukemia with maturation (M2), acute promyelocytic leukemia (M3), acute myelomonocytic leukemia (M4), acute monoblastic leukemia (M5a), acute monocytic leukemia (M5b), acute erythroid leukemia (M6), and acute megakaryoblastic leukemia (M7).

The distinction between M1 and M2 is based on the percentage of blasts in the bone marrow. M1 is diagnosed when >90% of nonerythroid marrow cells are myeloblasts, whereas M2 shows <90% myeloblasts in the bone marrow. The diagnosis of AML was required to have >3% of blasts positive for MPO. However, in those MPO-negative (or <3%) myeloid leukemia cases, the myeloid lineage can be identified by immunophenotyping or electron microscopic detection of MPO. These cases are now called AML with minimal differentiation (M0) (9,10). The incidence of M0 varies from 2% to 22% in different series (9–13). M0 is frequently associated with the presence of terminal deoxynucleotidyl transferase (TdT) (13).

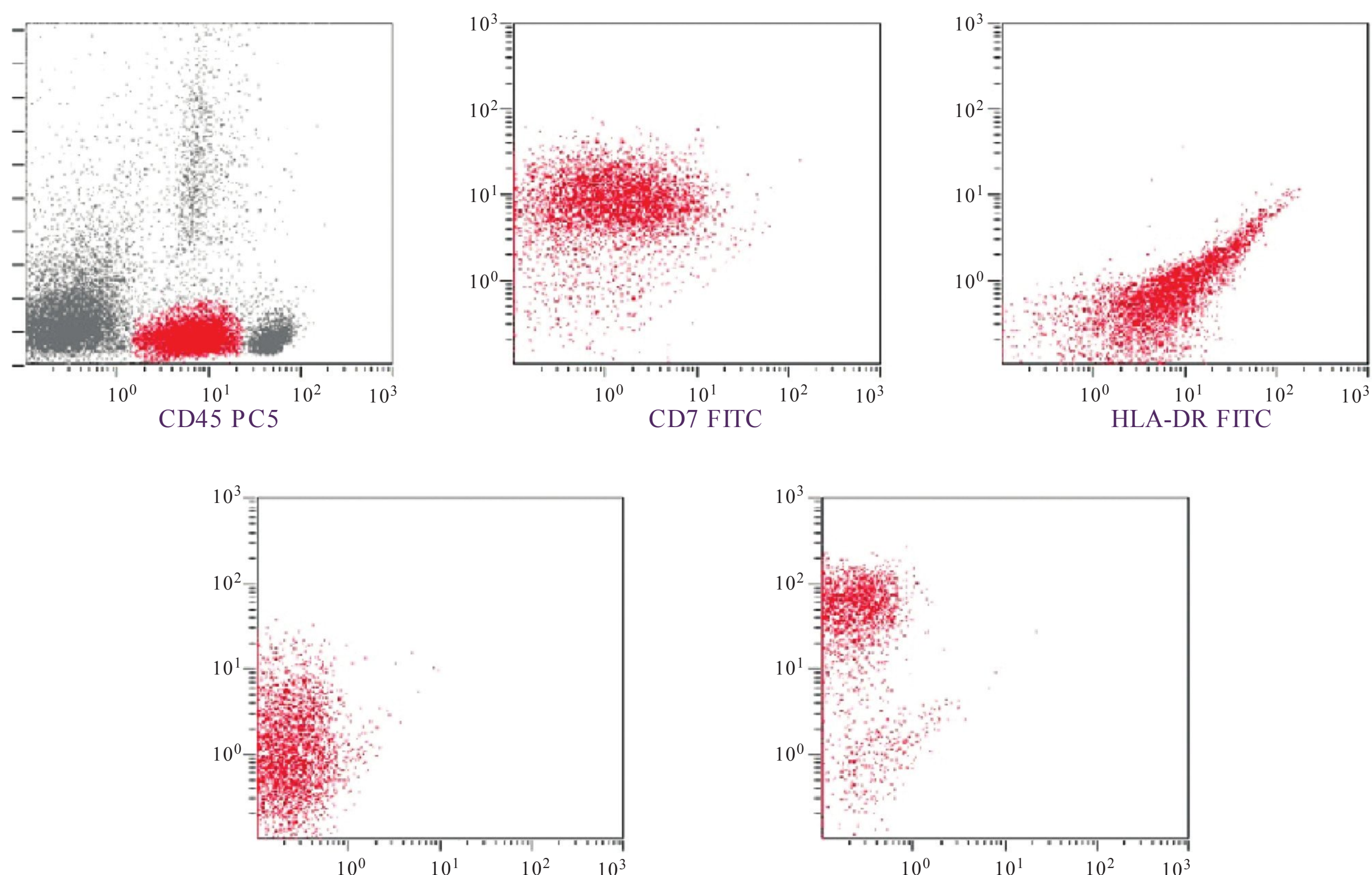


FIGURE 6.8.1 Flow cytometric histograms of the bone marrow show dual CD13-CD33/CD7 staining, with positive HLA-DR, CD117, and CD34. SS, side scatter; PC5, phycoerythrin-cyanin 5; FITC, fluorescein isothiocyanate; RD1, rhodamine; PE, phycoerythrin.

The 2008 World Health Organization (WHO) classification divides AML into seven categories: AML with recurrent genetic abnormalities; AML with myelodysplasia-related changes; therapy-related neoplasms; and AML, not otherwise categorized; myeloid sarcoma; myeloid proliferations

related to Down syndrome; and blastic plasmacytoid dendritic cell neoplasm (Table 6.8.1) (14–18). The FAB entities are now included in the category of AML, not otherwise categorized. One of the major basic changes in this classification is lowering the diagnostic threshold of blast count from 30% to 20%, because recent studies have indicated that patients with 20% to 30% blasts have a prognosis similar to that of patients with >30% blasts.

The new classification emphasizes the importance of clinical correlation of the AML entities, particularly the correlation of prognosis (18). Because cytogenetic abnormalities, multilineage dysplasia, and chemotherapy and/or radiation therapy have proved to be intimately related to prognosis in AML patients, they are established as new categories in the WHO classification. The rationale of creating new entities in the AML classification is explained in detail in a review article by Vardiman et al. (18).

Cytochemistry

Routine cytochemical stains for the study of AML cases include MPO (Fig. 6.8.4), specific esterase (e.g., CAE), and nonspecific esterase (e.g., NBE) (14,19,20). The two esterases can be stained simultaneously (combined esterase stain) (Fig. 6.8.5), so that two blood or bone marrow smears are usually sufficient for a routine cytochemical study.

MPO is usually strongly positive in M2, M3, M4, and M6; weakly positive in M1; and weakly positive or negative in

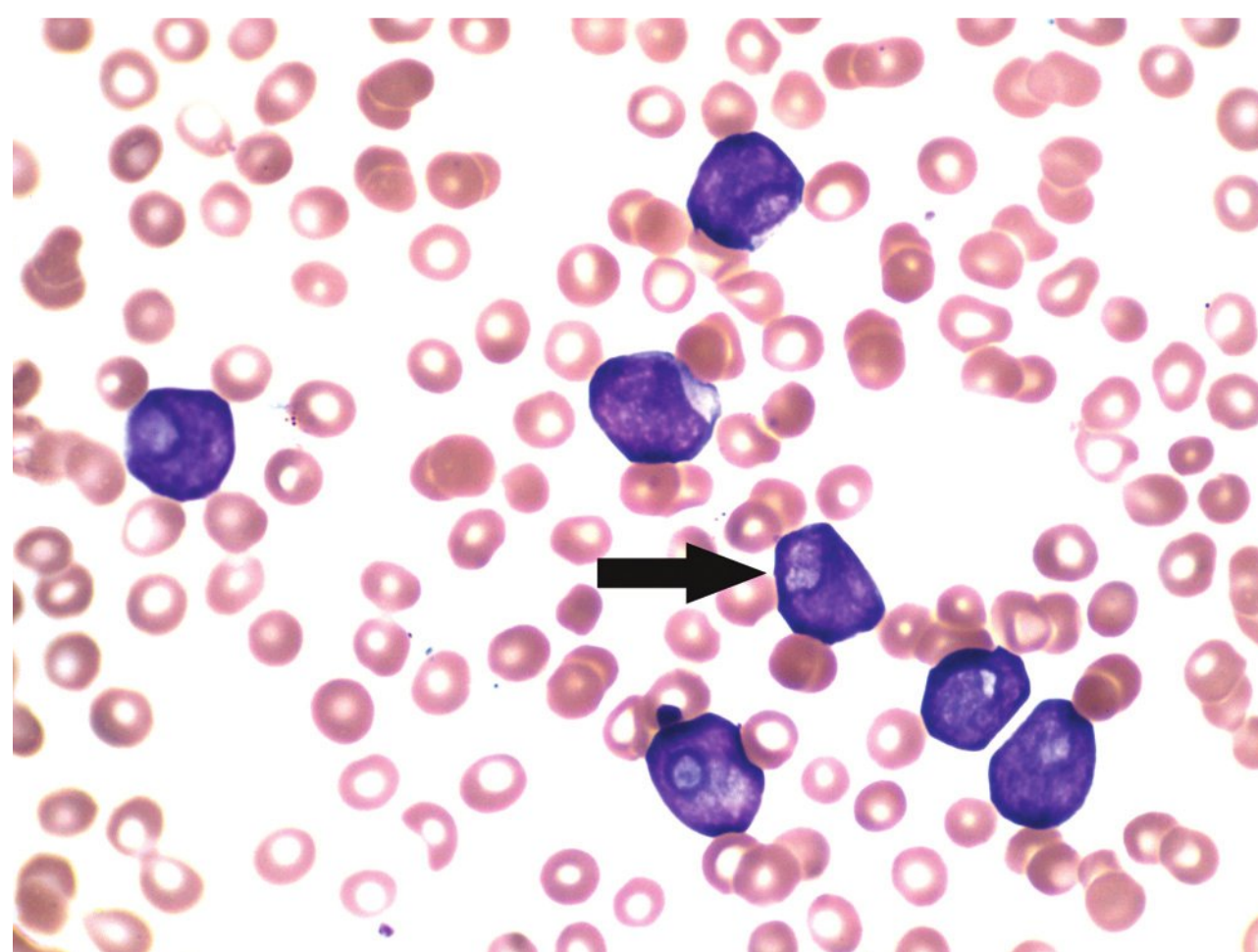


FIGURE 6.8.2 Peripheral blood smear shows seven myeloblasts with cup-like nuclei. The arrow points at the depression of a cup-like nucleus. Wright-Giemsa, 100× magnification.

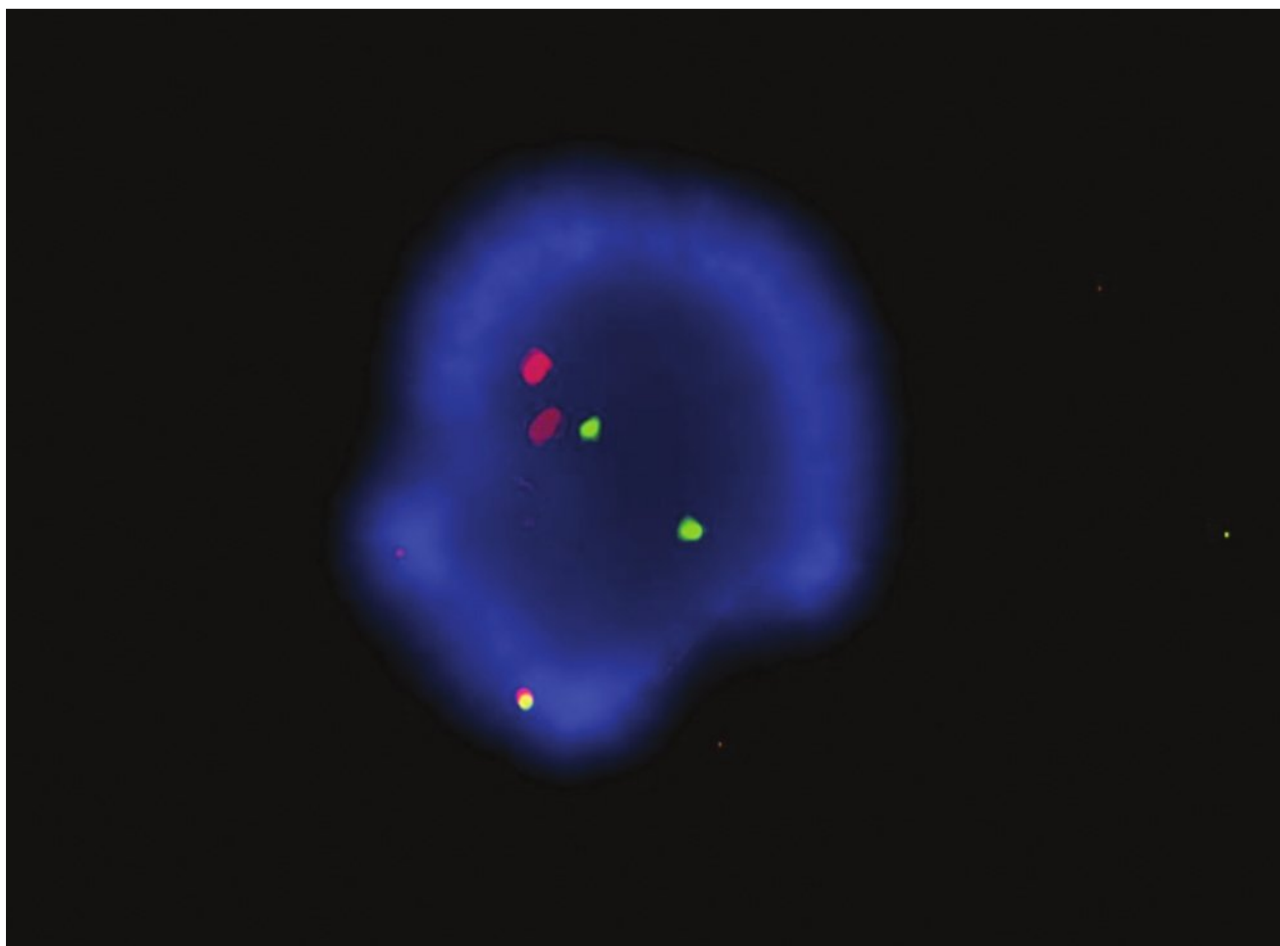


FIGURE 6.8.3 Fluorescence in situ hybridization with RARA and PML probes on a peripheral blood smear from the case of Figure 6.8.2. The blast shows two pairs of the normal green and orange signals. In addition, there is a fusion signal at the lower end of the nucleus due to trapping of both probes in the concavity of the cup-like nucleus. This phenomenon is seen in many cells of the same smear. Some cells reveal multiple fusion signals.

M5, but negative in M7. The peroxidase in megakaryocytes can be demonstrated only by electron microscopy, and is called platelet peroxidase. The eosinophilic peroxidase is characterized by its resistance to cyanide. The basophils are negative for peroxidase.

MPO-deficient neutrophils are found in about 40% of AML cases. These MPO-deficient neutrophils frequently disappear during complete remission and reappear during relapse (21). MPO deficiency and a low level of MPO activity in AML usually mean a poor prognosis (22).

Sudan black B stain has the same reaction as MPO to various leukocytes. In a study of 1,386 cases of AML, the Medical Research Council of England found that increased Sudan black B positivity predicted a high remission rate and long survival and suggested that >50% of blast with Sudan black B positivity should be used to distinguish M2 from M1 (23).

NBE or other nonspecific esterase (e.g., α -naphthyl acetate esterase) is positive for the monocytic series, and CAE or other specific esterase is positive for the myelocytic series (19). However, about 13% to 37% of promyelocytic leukemia cases may show strongly positive nonspecific esterase (24). A subset of myelomonocytic leukemia displays double staining of specific and nonspecific esterases in all blasts. Another study shows that all types of AML may show double staining in some cases (25).

The periodic acid-Schiff (PAS) stain showing a block pattern is seen in most cases of acute lymphoblastic leukemia (ALL). However, negative PAS staining does not rule out ALL, and positive PAS staining can be seen in occasional cases of AML (19). PAS is probably more useful in distinguishing normal erythroblasts from leukemic erythroblasts. In erythroleukemia, the pronormoblasts and other stages of normoblasts can be positive, but normal

TABLE 6.8.1

World Health Organization Classification of AML

AML with recurrent genetic abnormalities

- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13q22) or t(16;16)(p12;q32); CBFB-MYH11
- Acute promyelocytic leukemia with t(15;17)(q22;p22); PML-RAR α
- AML with t(9;11)(p22;q23); MLLT3-MLL
- AML with t(6;9)(p23;q34); DEK-NUP214
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); RPN1-EVI1
- AML (megakaryoblastic) with t(1;22)(p13;q13); RBM15-MKL1
- AML with mutated NPM1 (provisional)
- AML with mutated CEBPA (provisional)

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasms

AML not otherwise categorized

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemias
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome

- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

nucleated erythrocytes are PAS negative. It should be cautioned, however, that nucleated erythrocytes can be PAS positive in myelodysplastic syndrome (MDS). In megakaryoblastic leukemia, a peripheral PAS staining pattern of megakaryoblasts is characteristic.

Acid phosphatase (AP) staining can be seen in all leukocytes, but tartrate-resistant AP positivity is relatively specific for hairy cell leukemia (19). A focal paranuclear AP stain is characteristic of T lymphocytes and T lymphoblasts. However, in myeloid series, the AP staining is stronger and diffuse. Therefore, AP can be used, but is not particularly helpful in differential diagnosis between AML and ALL.

With the development of flow cytometry and immunohistochemistry, cytochemical staining is no longer essential in the diagnosis of AML. However, in difficult cases of

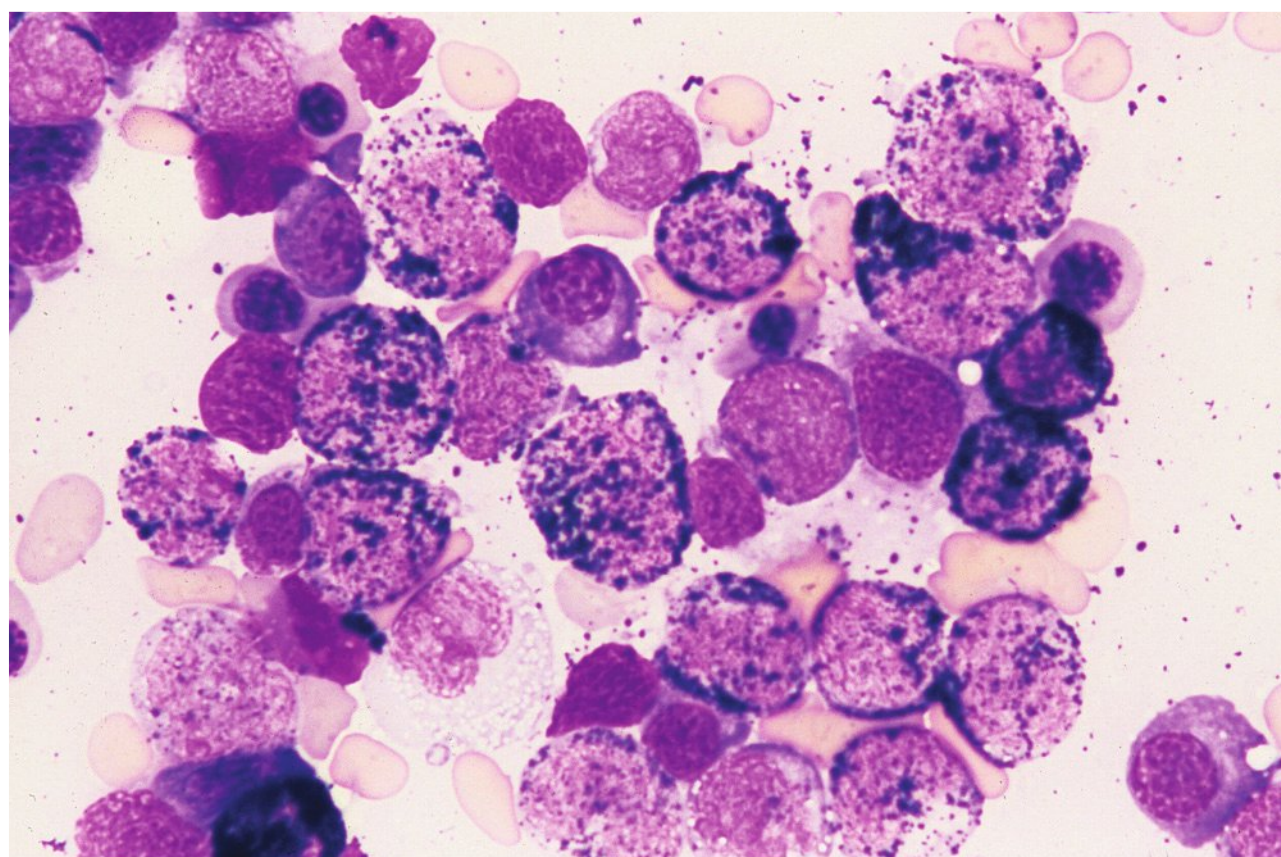


FIGURE 6.8.4 MPO stain of the bone marrow shows positive staining in several myeloblasts as well as maturing myeloid cells. 100× magnification.

AML-M4 and AML-M5, the esterase stains are still superior to other techniques in defining the cell lineage with direct morphologic correlation. Unfortunately, cytochemical staining is technically difficult and cannot be done with automated instruments. Furthermore, because of the lack of demand, most commercial or reference laboratories do not offer cytochemical services.

Morphology

In the current case, the bone marrow aspirate showed that >90% of nonerythroid cells were blasts with scanty cytoplasm, immature chromatin pattern, and prominent nucleoli (Fig. 6.8.6). A few of them contained Auer rods in the cytoplasm, and a few cytoplasmic granules were occasionally seen. These features are consistent with myeloblasts. The core biopsy revealed diffuse infiltration of immature myeloid cells replacing the normal hematopoietic cells (Fig. 6.8.7). The same blasts were also found in the peripheral blood smears (Fig. 6.8.8).

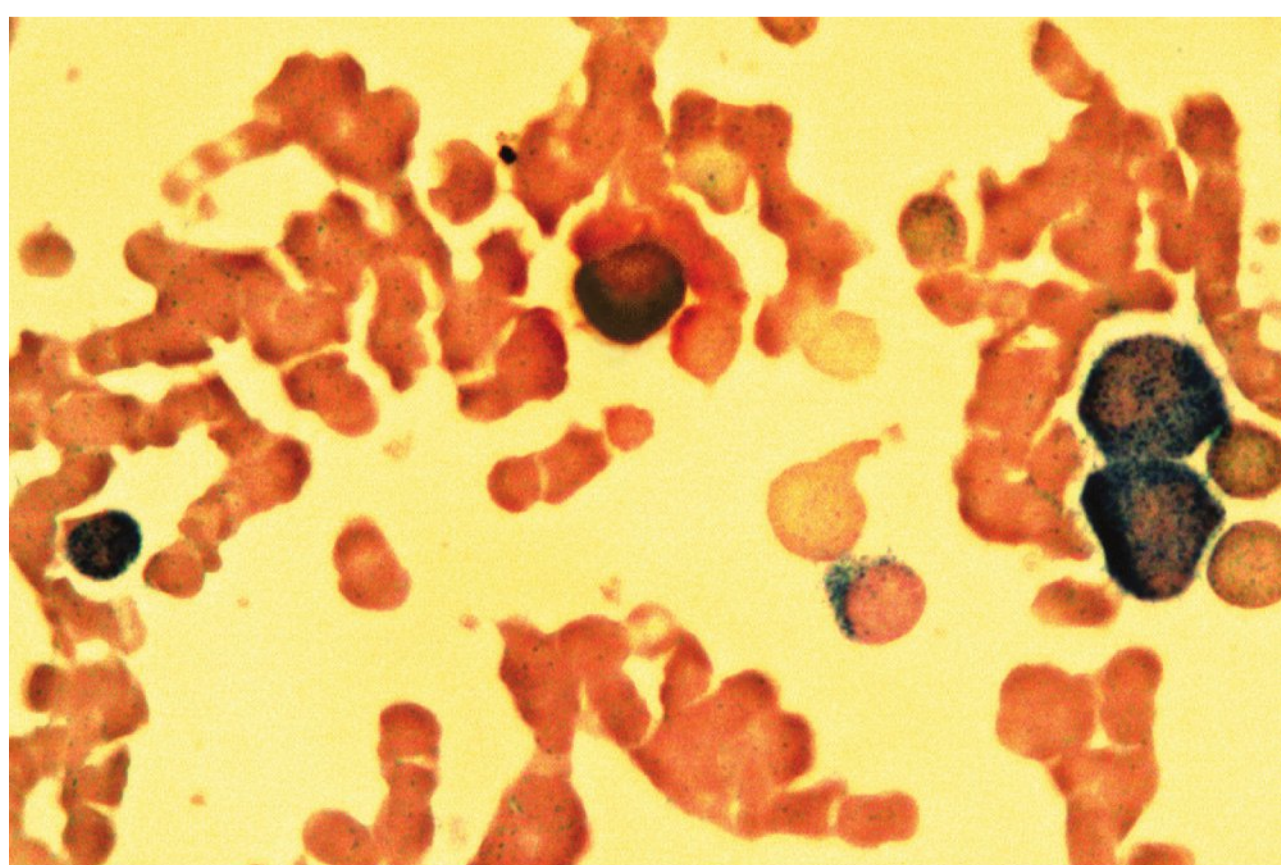


FIGURE 6.8.5 Combined esterase stain reveals CAE staining (blue) in two myeloblasts and one granulocyte, and a-naphthyl butyrate staining (brown) in a monocyte. 100× magnification.

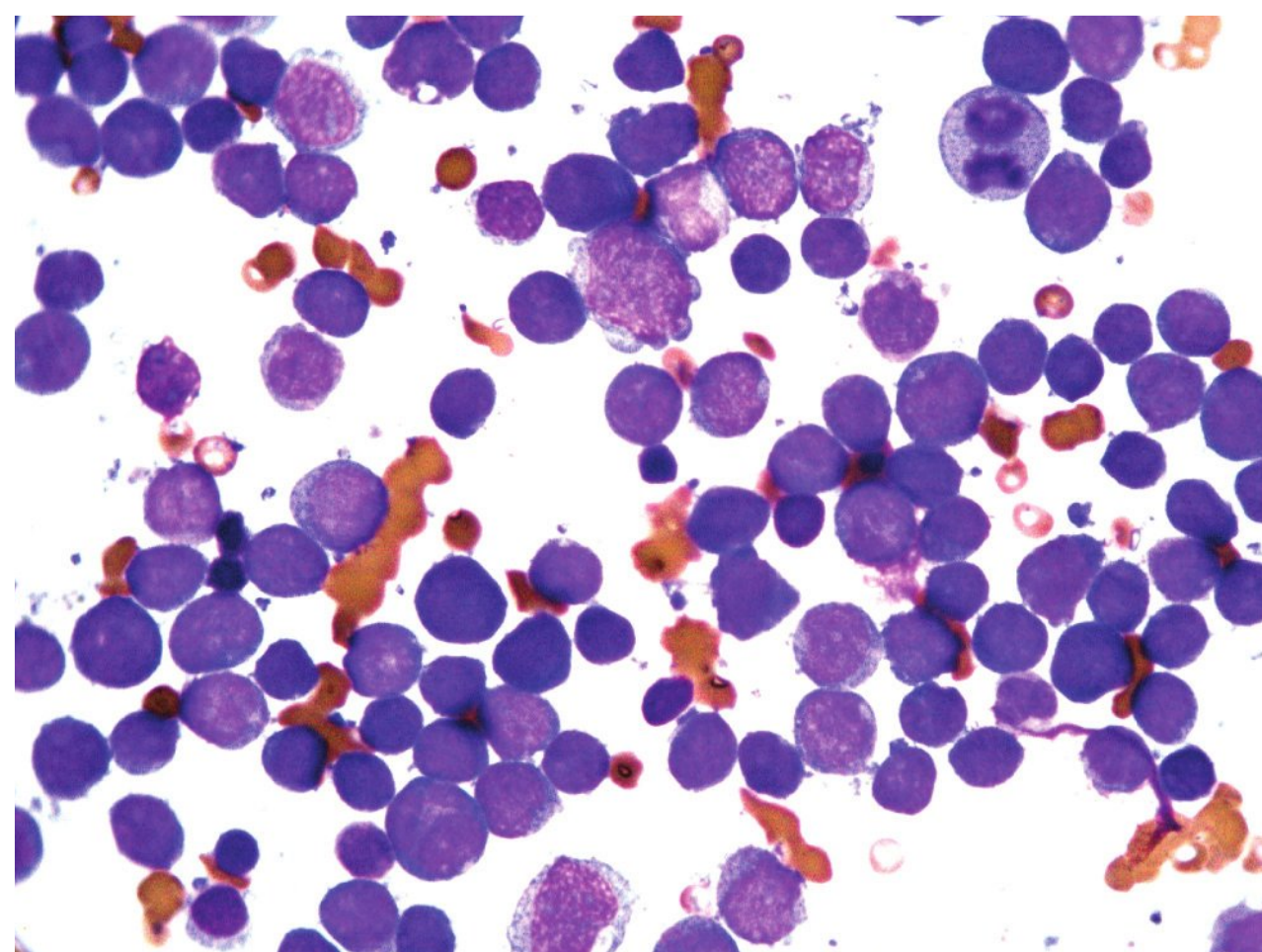


FIGURE 6.8.6 Bone marrow aspirate reveals almost exclusively blasts in the marrow with a high nuclear/cytoplasmic ratio, immature chromatin pattern, and nucleoli. Wright–Giemsa, 60× magnification.

The leukemic nature in this case is determined on the basis of extensive infiltration of the bone marrow in the core biopsy and the presence of >20% blasts and <10% nonblastic myeloid cells in the marrow aspirate. Cytochemistry demonstrated positive staining for MPO in about 3% of myeloblasts and CAE in 30% of blasts. The NBE stain was negative for the immature cells. These results confirmed the morphologic impression of myeloblastic leukemia. The flow cytometric result also confirmed the myelocytic lineage by the demonstration of 86% CD13-CD33-positive cells. The percentage of MPO was low and can be considered partial deficiency. In some myeloid leukemia cases, the percentage of CD13-CD33 is low but that of MPO is high. That is why both of these myeloid markers should be included in flow cytometric studies of AML. The presence of high percentages of CD34- and CD117-positive cells and

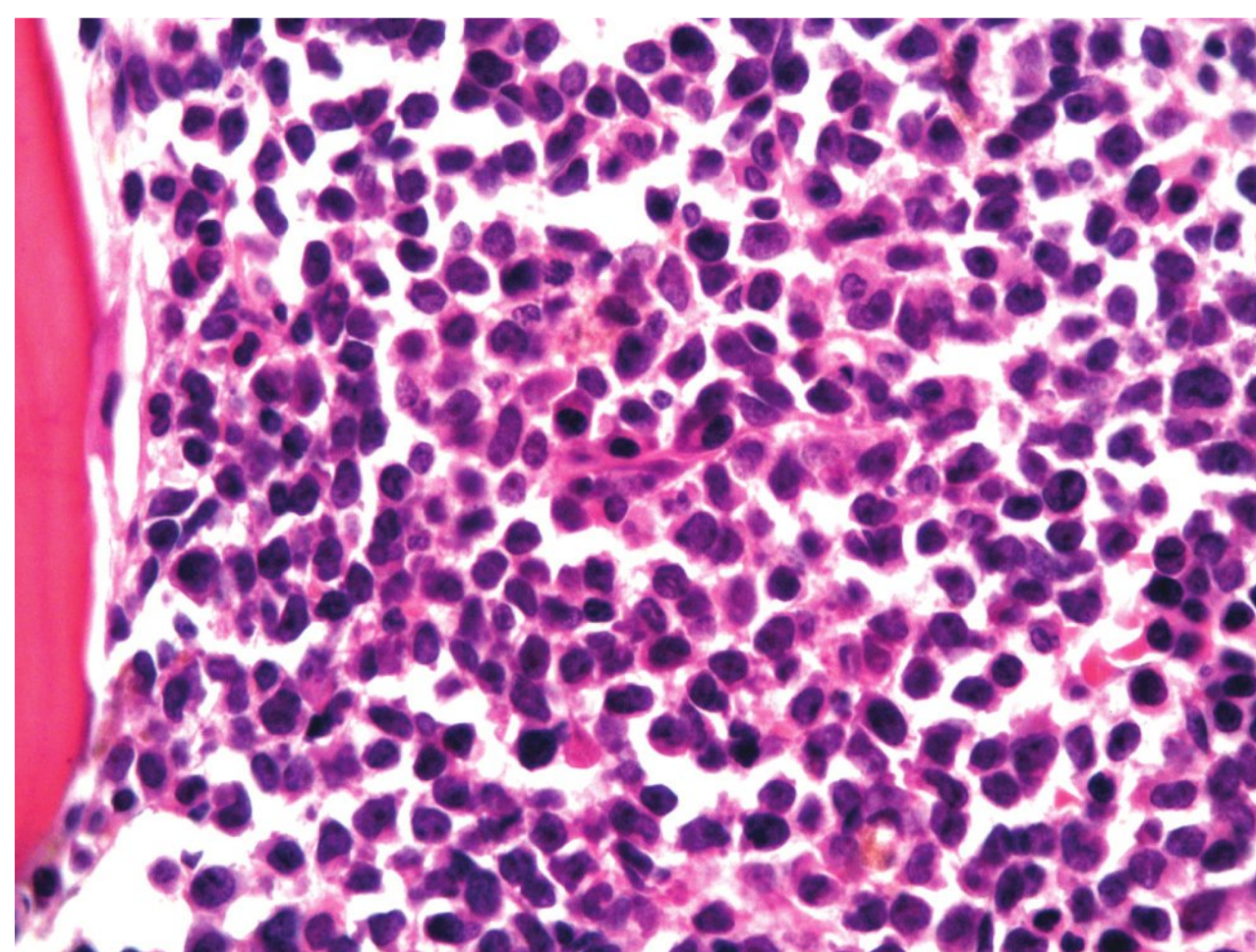


FIGURE 6.8.7 Bone marrow biopsy shows 100% cellularity, and normal hematopoietic cells are almost totally replaced by blastic cells. Hematoxylin–eosin, 60× magnification.

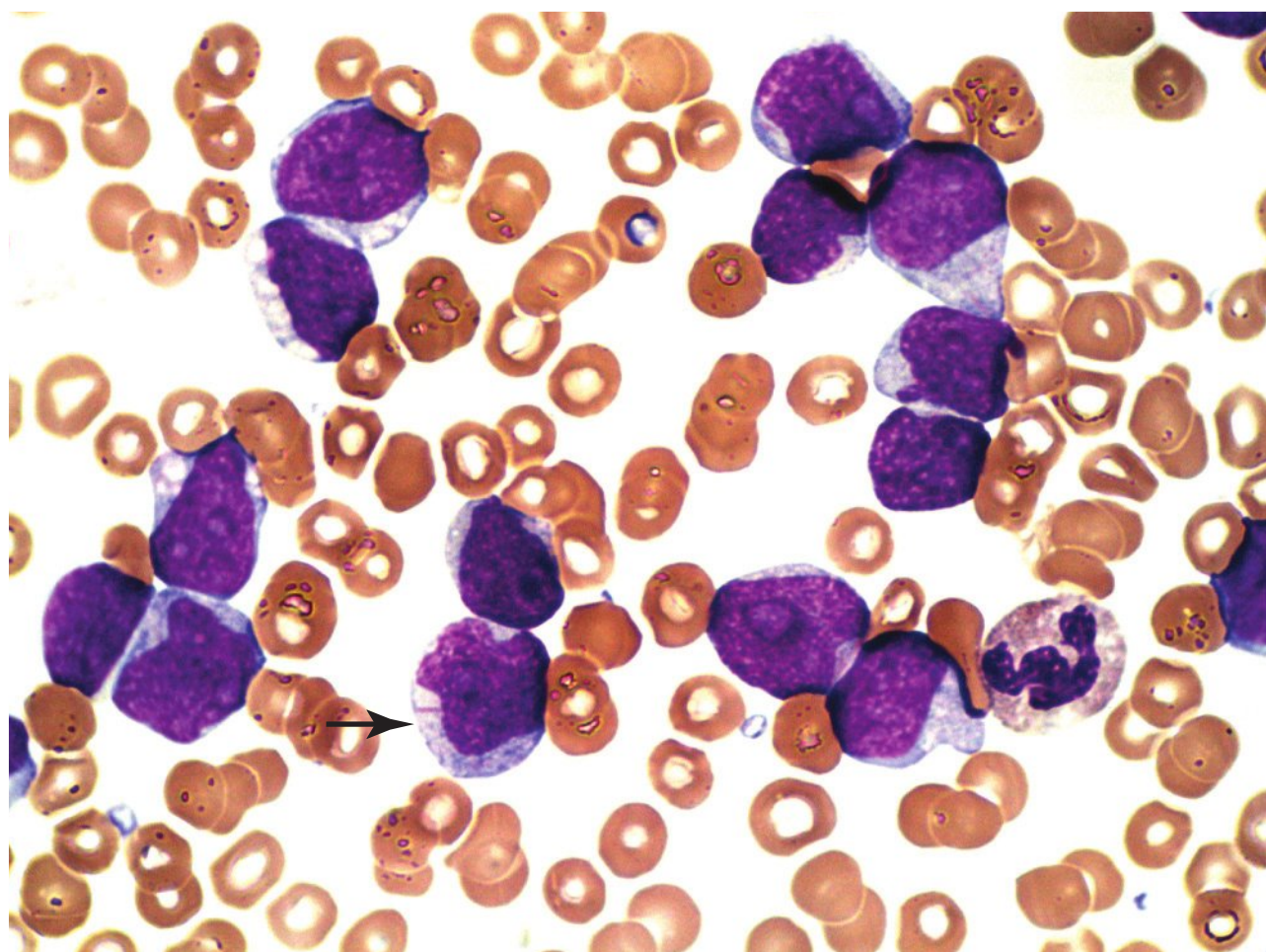


FIGURE 6.8.8 Peripheral blood smear shows multiple myeloblasts with similar features as those seen in the bone marrow. One Auer rod in the cytoplasm of a blast is indicated (arrow). Wright–Giemsa, 100× magnification.

the dual CD13-CD33/CD7 staining are supportive of a malignant cell population, which will be discussed in the immunophenotype section. With all this information, a diagnosis of AML without maturation (M1) was established.

The major differential diagnosis for AML is ALL. Myeloblasts and lymphoblasts can be distinguished by their chromatin pattern, number and prominence of the nucleoli, amount of cytoplasm, and presence or absence of cytoplasmic granules (see Table 6.17.2). Nevertheless, all these morphologic criteria are not absolute: The only reliable morphologic marker is the Auer rod, which, however, is present in the myeloblasts in only 21% of AML cases (26). Therefore, flow cytometry and cytochemistry are needed to help with the diagnosis.

Immunophenotype

As mentioned before, cytochemical staining can be totally negative in AML, and sometimes the staining is difficult to interpret or inconclusive. Therefore, immunophenotyping

is most useful in substantiating the diagnosis (14,20,27,28). There is also evidence that immunophenotypes are frequently reliable predictors for prognosis and sensitive markers for detecting minimal residual disease (29). In addition, immunophenotyping may identify mixed lineage phenotypes, but the clinical significance of these phenotypes is controversial.

Terstappen et al. (30) found that AML cells may differ from normal cells in several aspects: expression of non-myeloid antigens (e.g., CD2, CD5, and CD7), asynchronous expression of myeloid-associated antigens (e.g., coexpression of CD34 and CD15), overexpression of myeloid-associated antigens (e.g., CD14 and CD34), and absence of expression of myeloid-associated antigens (e.g., CD33, CD11b, CD15). Because selective loss of certain myeloid antigens is helpful in distinguishing leukemia from benign myelocytosis, the use of mixed antibodies (e.g., CD13-CD33) for screening purposes may mask this phenomenon.

Neame et al. (11) recommended the use of seven monoclonal antibodies (CD33, CD13, CD14, CD15, HLA-DR, AML2.23, and polymorphonuclear neutrophil 6/29) for immunophenotyping, which can help to distinguish the first five types of AML. In our experience, the first five antibodies should be sufficient for differential diagnosis (Table 6.8.2). Essentially, M1 and M5 are all positive for CD33 and CD13, whereas M4 and M5 are also positive for CD14 (Mo2 or My4) and CD64, the monocyte markers. M3 is characterized by the low percentage or complete absence of HLA-DR, which is positive for the blasts but negative for the promyelocytes. The distinction between M1 and M2 is the fact that there is a negative reaction to CD15 in M1, but a positive reaction to CD15 in M2 through M5. The above mentioned are typical immunophenotypes present in most cases, but exceptions are seen from time to time. CD15 can also be positive in M1 and is now seldom used for differential diagnosis.

Immunophenotyping of M6 depends on a positive reaction to CD71, glycophorin, or hemoglobin A. The first two are demonstrated by flow cytometry, while glycophorin and hemoglobin A by immunohistochemistry. M7 can be identified by positive reactions to CD41 and CD61 but negative reactions to CD42 (see Case 13). The reaction in M6

TABLE 6.8.2							
Correlation of Immunophenotyping and FAB Classification (AML, NOS)							
Antigen	M1	M2	M3	M4	M5	M6	M7
CD33	+	+	+	+	+	±	+
CD13	+	+	+	+	+	–	–
CD14/CD64	–	–	–	+	+	–	–
CD15	–	+	+	+	+	±	–
HLA-DR	+	+	–	+	+	±	±
CD41/CD61	–	–	–	–	–	–	+
Glycophorin/ Hemoglobin A	–	–	–	–	–	+	–

and M7 cases to other myelomonocytic markers is variable and is not dependable for their identification.

The Morphologic, Immunologic and Cytogenetic Cooperative Study Group includes CD34 and CD11 in the phenotyping panel to distinguish M1 from other subtypes of AML (3). As mentioned before, CD11b and CD11c are helpful in identifying monocytes. CD34 is now used as an integral component of the AML panel.

Normally, CD34 is present only on stem and/or progenitor cells, but it is expressed in 40% of AML cases (31). Therefore, CD34 helps to distinguish AML from benign myeloproliferative disorders. Although CD34 may also be detected in cases of MDS, its percentage is usually lower than that in AML cases. When a high percentage is present in a case of MDS, it predicts leukemic transformation (32). CD34 is found more frequently in M0, M1, and M5a (33,34) but is often absent in M3 (35). It is associated with either good or poor prognosis, depending on the cytogenetic alteration in a particular case and the cell lineage (31). Generally, it predicts poor prognosis in AML but good prognosis in ALL (34). When CD34 is related to poor prognosis, it is usually due to the correlation between CD34 and the multiple drug resistance (MDR) protein (31). CD34 may appear at relapse of CD34-negative AML, supporting its being an unfavorable marker (34). The use of CD34 for blast count is discouraged (18), because not all blasts express CD34 and CD34 can also be detected on dysplastic and immature myeloid cells other than myeloblasts.

A relatively new marker for myeloid lineage is CD117 (c-kit or stem cell factor receptor) (36–38). This antigen also marks the immature cells so that it can help to distinguish benign myeloid proliferation and myeloid leukemia. CD117 is better than CD34 as an immature cell marker in two aspects. First, CD117 is highly lineage specific; it has been found in lymphoid leukemia or lymphoma only in occasional cases (30–32). Second, it can be demonstrated in M3 cases, which usually show negative CD34. CD117-positive AML cases generally carry a favorable prognosis (20). Being a tyrosine kinase, CD117 is a potential target for tyrosine kinase inhibitor treatment.

Lymphoid markers are not infrequently identified in AML cases. As will be mentioned later, its presence may denote specific subtypes of AML. The important lymphoid marker for AML is CD7, which is included in our routine AML panel. CD7 is not present on normal myelomonocytic cells, but is detected on leukemic cells in 9.4% to 37.5% of five AML subtypes (M0, M1, M2, M4, and M5) (39,40). Therefore, the demonstration of dual CD7 and CD13-CD33 staining is consistent with AML. CD7-positive AML cases more frequently express the myeloid progenitor-associated antigens, such as CD34, HLA-DR, and TdT than do CD7-negative AML cases (39). This finding may suggest phenotypic immaturity of CD7+ AML and probably explains why none of the promyelocytic leukemia (M3) cases studied showed positive CD7 (34,39,40). A few studies of CD7+ AML showed that patients with this phenotype were younger, predominantly male, had more frequent involvement with the liver and central nervous system, and responded poorly to standard chemotherapy (39).

Two other special lymphoid markers that are present in AML cases are CD2 and CD19. CD2 is frequently associated with M3 and M4Eo, while CD19 with M2 subtype (34,41). In one study of 170 cases of AML, CD2 and/or CD19 were detected in 33% of cases and were associated with good prognosis (42). In another study, CD19 expression was associated with poor prognosis (43). In a third study, CD20 was found to be the most commonly found lymphoid marker in AML cases, but it often was expressed in only a subpopulation of leukemic cells (44).

Previously, the presence of a lymphoid marker on AML cells was considered biphenotypic or mixed lineage leukemia. However, because lymphoid markers are so frequently encountered on AML cells, the presence of a single lymphoid marker no longer constitutes a diagnosis of mixed lineage or biphenotypic leukemia. There is still not a universal criterion to denote a biphenotypic or bilineage leukemia. Some authors consider two or more markers of another lineage as the criterion (45); others use a scoring system based on different combinations of B lineage, T lineage, and myeloid antigens (46). Antigens can also be generated after in vitro culture, leading to a bilineage phenotype (45). Therefore, the significance of identifying a biphenotypic population is still not conclusive. Some studies suggested, however, that the AML with lymphoid markers responded well to ALL therapy and thus convey a better prognosis (42). In addition, when lymphoid markers are present, immunoglobulin genes or T-cell receptor genes may be rearranged (42,47,48). The 2008 WHO classification includes a new entity of acute leukemia of ambiguous lineage, which is discussed in Case 16 (49).

TdT is demonstrated in about 18% of AML cases (children 19%, adults 21%) (50,51). The percentage of positive cells is usually lower in AML than in ALL. TdT positivity is more common in the immature subtypes of AML (M0 and M1) and is frequently associated with CD34, another immature marker. Although some early reports considered a direct correlation between TdT positivity and immunoglobulin or T-cell receptor gene rearrangement, this finding is not supported by subsequent studies. Therefore, TdT should be viewed as an immature cell marker, but it is not lineage specific. The prognostic significance of the presence of TdT marker is controversial.

CD45, a panleukocyte antigen, is present in normal and leukemic myeloid cells. However, its low molecular isoform, CD45RO, is expressed in normal cells, whereas its high molecular isoform, CD45RA, is expressed almost exclusively in AML cases with or without coexpression of CD45RO (52).

CD56, a natural killer-cell marker, is present in various subtypes of AML. In a study of 80 bone marrow specimens, CD56 was found in 15% M0, 22% M2, 17% M3, 67% M4, and 100% M5 cases (35). There is a unique subtype of CD56+ AML with a phenotype of CD56+, CD33+, CD13±, CD34-, HLA-DR-, CD16-. This subtype is characterized by a high white blood cell count and marked nuclear folding with variable cytoplasmic granularity resembling microgranular M3 (M3v) (53). The authors designated these cases as myeloid/natural killer-cell acute leukemia. However, the 2008 WHO classification considers these cases as AML until further evidence proves otherwise (49).



TABLE 6.8.3		
Correlation of Cytogenetic and Molecular Abnormalities with FAB Classification		
Cytogenetic abnormalities	Genes involved	FAB type
	Gene activation	
inv(3)(q21q26)	Ribophorin 1/EVI1	M0, M1, M2, M4, M5, M6, M7
t(3;3)(q21;q26)	Ronphorin 1/EVI1	M1, M2, M4, M6
	Gene fusion	
t(1;22)(p13;q13)	N-RAS/C-SIS	M7 (infantile)
t(6;9)(p23;q13)	DEK/CAN	M1, M2, M4
t(7;11)(p15;p15)	HOXA9/NUP98	M2, M4
t(8;16)(p11;q13)	MOZ/CBP	M5b/M4
t(8;21)(q22;q22)	RUNX1/RUNX1T1 (AML1/ETO)	M2
t(9;11)(p22;q23)	AF9/MLL	M4, M5
t(10;11)(p12;q23)	AF10/MLL	M4, M5
+11	ALL1/MLL	M1, M2
t(11;17)(q23;q21)	MLL1/AF17	M5
t(11;19)(q23;p13.1)	MLL1/ELL	M4, M5
t(11;19)(q23;p13.3)	MLL1/ENL	M4, M5
t(15;17)(q22;q11-12)	PML/RARa	M3
inv(16)(p13q22)	MYH11/CBFb	M4Eo
t(16;16)(p13;q22)	MYH11/CBFb	M4Eo
t(16;21)(p11;q22)	FUS/ERG	M1, M2, M4, M5

FAB, French-American-British; EVI 1, ecotropic viral integration site 1; N-RAS, an oncogene derived from rat sarcoma virus; C-SIS, simian sarcoma oncogene; HOXA, homeobox A; MOZ, monocytic leukemia zinc finger; CBP, CREB-binding protein; ETO, eight twenty one; AML, acute myeloid leukemia; MLL, mixed lineage leukemia; ALL, acute lymphoblastic leukemia; PML, promyelocytic leukemia; RAR, retinoic acid receptor; MYH, smooth muscle myosin heavy chain; CBF, core-binding factor.

Comparison of Flow Cytometry and Immunohistochemistry

In general, flow cytometry is preferred to immunohistochemistry mainly because more monoclonal antibodies are available for the former technique. It is difficult to subclassify AML, particularly for M4 and M5, with the latter technique (54). In these cases, cytochemical staining is the preferred technique. Previously, the detection of CD34 and CD117 by flow cytometry was much more sensitive than by immunohistochemistry. Recently, however, the quality of antibodies used for immunohistochemical stains of CD34 and CD117 has been greatly improved, so that immunohistochemistry can be reliably used for identification of myeloblasts and monoblasts.

Molecular Genetics

In recent years, most progress in AML has been made on the molecular genetic front. With the improvement of cytogenetic techniques, clonal chromosome abnormalities can be detected in 55% to 78% of cases of adult AML and in

79% to 85% of childhood AML (55). In these cases, >30 different structural abnormalities, including translocations, deletions, and inversions, have been repeatedly implicated as primary nonrandom chromosome rearrangements (Table 6.8.3) (55–57). In contrast to lymphomas, which frequently show complex karyotypes with multiple aberrations, AML often reveals only one chromosome abnormality. Most of these aberrations are leukemia specific because they are not found in nonhematologic neoplasms. Therefore, these abnormalities are considered to represent primary chromosome changes. Additional cytogenetic abnormalities may appear during the clinical course, frequently at the time of relapse. These additional aberrations are called secondary chromosome abnormalities, which are believed to contribute to disease progression. Primary aberrations often involve structural changes (e.g., reciprocal translocations and inversions), whereas the secondary aberrations usually involve genomic imbalances (trisomies, monosomies, deletions, and unbalanced translocations).

Some of these cytogenetic abnormalities are so specific that they define distinct subtypes of AML, regardless of the blast count (14). These abnormal karyotypes correlate well with the FAB classification. The most striking examples are the association of t(15;17) with M3, inv(16) or t(16;16) with M4Eo, t(8;21) with M2, t(1;22) with infantile M7, and t(8;16) with M4 or M5b with erythrophagocytosis by leukemic cells. However, only t(8;21), t(15;17), inv(16), and t(16;16) are specific enough to disregard the blast count for the diagnosis of AML (18). In the 2008 WHO scheme, t(9;11), t(6;9), inv(3), t(3;3), and t(1;22) are newly added as distinct clinical entities (17). AML with NPM1 and AML with CEBPA are included as provisional entities (17).

Cytogenetic abnormalities also are accurate predictors for prognosis in AML. It is generally accepted that t(15;17), t(8;21), and inv(16) are associated with favorable prognosis (17,18,58). Cases with t(9;11), gains of whole chromosomes or loss of the Y chromosome are associated with intermediate risk, while cases with t(6;9), inv(3)/t(3;3), 5q-, 7q-, and complex karyotype predict poor prognosis (58,59). Some karyotypes are associated with certain lymphoid or other surface markers (33,35). The expression of CD2 is associated with M4Eo/inv(16), CD19 is associated with M2/t(8;21), and absence of CD34 and HLA-DR is associated with M3/t(15;17).

Many genes are now known to be converted into leukemia genes by the mechanism of either gene activation or gene fusion (56). Gene activation occurs when a translocated gene is under the control of a new promoter and/or enhancer. This activated or deregulated gene then becomes an oncogene, leading to leukemogenesis. Gene fusion occurs when segments from two different genes are fused together to give rise to a chimeric transcript. The transcript is then translated into chimeric proteins that lead to leukemogenesis through the transduction system. In AML, most karyotypic changes result in gene fusion with only a few abnormalities resulting in gene activation.

The recently advocated two-hit theory hypothesizes that leukemogenesis of AML requires two classes of mutation (58,60). The class I mutations, including FLT3, KIT, NRAS/KRAS, and PTPN11, confer a proliferation and survival advantage and play a role in aberrant activation of signal transduction pathways. The class II mutations, including CEBPA, NPM1, and some recurrent chromosomal translocations/inversions (t[8;21], inv[16], t[16;16], and t[15;17]), lead to a halt in differentiation via interference with transcription factors or coactivators.

Despite the advances in cytogenetic techniques, a proportion of submicroscopic alterations of genetic material can only be detected by molecular techniques. These conditions are called cryptic abnormalities (61). The most frequently identified mutations in AML cases with normal karyotypes include FLT3-ITDs, FLT3 tyrosine kinase domain mutations, MLL partial tandem duplications, and a variety of nucleotide-substitution mutations or short insertion or deletion mutations within the coding region of the NPM1, CEBPA, NRAS, and WT1 genes. The NPM1 and CEBPA mutations confer a favorable prognosis, while the FLT3-ITD and MLL-PTD are the opposite (7,58,62). However, if NPM1 is coexistent with FLT3-ITD, the prognosis will become

adverse. The prognostic value of NRAS and WT1 remains unclear at this stage. The NPM1 mutation and FLT3-ITD are the most commonly mutated genes in AML cases with a normal karyotype, accounting for 45% to 55% and 35% to 45% of cases, respectively (62,63).

There are also conditions that the cytogenetic results are false negative due to technical problems. In two large study series, reverse transcription–polymerase chain reaction (RT-PCR) detected chimeric CBF α 2/ETO (core-binding factor/eight twenty one) and CBF β /MYH11 (core-binding factor/smooth muscle myosin heavy chain) transcripts in one third of patients with no detectable cytogenetic abnormalities (64,65). Even in acute promyelocytic leukemia, 15% of cases were found to be cytogenetically negative and molecularly positive in one study (66).

In about 40% of AML cases, no cytogenetic or molecular abnormalities are detected (50). However, the newly used gene expressing profiling (GEP) technique may gradually fill this gap. GEP may help distinguish AML from ALL (67,68). It may also demonstrate specific profiles in various cytogenetic subtypes of AML, such as t(8;21), inv(16), t(15;17), and MLL chimeric fusion genes (69–71). In limited studies, GEP has been shown to be able to stratify AML cases with or without cytogenetic abnormalities and to predict the prognosis in these subsets; these are examples of some very attractive applications of this new technology (59).

The salient features for laboratory diagnosis of AML-M1 are summarized in Table 6.8.4.

Clinical Manifestations

Clinical symptoms are mainly due to the failure of the leukemic cells to mature and to the inhibition of normal hematopoiesis. Most patients may have anemia and/or thrombocytopenia. As a result, these patients have symptoms of fatigue, malaise, weakness, or hemorrhages. When the mature granulocytes are markedly decreased, superimposed infections are a common phenomenon. A fungal infection can be fatal to the patient.

AML has a bimodal age distribution. The first group, de novo AML, is usually seen in children and young adults with chromosomal abnormalities (mostly translocation) (57). The second group (secondary AML) is seen in elderly persons, associated with MDS, alkylating agent chemotherapy, or Fanconi anemia. It can also be seen in a subset of young patients.

The true de novo AML is characterized by a younger age group, absence of multistep progression, similar cytogenetic abnormalities, and, frequently, good response to chemotherapy. The second group, also called MDS-related AML, is characterized by resistant leukemia, poor marrow reserve with prolonged cytopenia after chemotherapy, early relapse, common cytogenetic abnormalities shared with MDS, and frequent multilineage dysplastic morphology in residual hematopoietic cells. Therefore, these two groups are different not only in age, but also in cytogenetic makeup, therapeutic response, and prognosis.

Adverse prognostic factors in patients with AML include unfavorable karyotype, age >60 years, secondary AML, poor performance score, features of multidrug

TABLE 6.8.4

Salient Features for Laboratory Diagnosis of AML without maturation

- 1. >90% myeloblasts in the bone marrow or peripheral blood
- 2. >3% MPO-positive blasts in the bone marrow
- 3. Blasts positive for CAE but negative for NBE
- 4. Blasts positive for CD33, CD13, HLA-DR
- 5. Blasts in most cases positive for CD117 or CD34
- 6. Blasts negative for CD14, CD15, CD64, CD41/CD61, glycophorin/hemoglobin A/CD71
- 7. Immunoglobulin or TCR gene rearrangement in mixed lineage leukemia
- 8. Possible cytogenetic aberrations: inv(3)(q21q26), t(3;3)(q21;q26), t(6;9)(p23;q34), +11, t(16;21)(p11;q22)

AML, acute myeloid leukemia; CAE, chloroacetate esterase; MPO, myeloperoxidase; NBE, a-naphthyl butyrate esterase; TCR, T-cell receptor.

resistance, leukocyte count >20,000/mL, unfavorable immunophenotype, CD34 positivity, and elevated lactate dehydrogenase levels (3,72). The identification of FLT3 mutations and overexpression of ecotropic viral integration site 1 (EVI 1) have been included recently as independent indicators for an unfavorable prognosis (20). In general, AML carries a worse prognosis than ALL. In adult AML cases, the cure rates are approximately 40% to 50%, as compared to the 75% to 80% cure rates in pediatric ALL cases (73). However, the current therapeutic protocols may achieve complete remission in 70% to 80% of AML patients (74). With the discovery of increasing numbers of pathogenic mutations, quantitation PCR techniques have played an increasingly important role in monitoring minimal residual disease, leading to more efficiently tailored treatments for AML patients (75).

REFERENCES

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the acute leukemias. *Br J Haematol*. 1976;33:451–458.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:620–629.
3. Second MIC Cooperative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukemias. *Br J Haematol*. 1988;68:487–494.
4. Mufti GJ, Bennett JM, Coasguen J, et al. diagnosis and classification of myelodysplastic syndrome: International Working Group on Morphology of myelodysplastic syndrome (IWGM-MDS) consensus proposals for the definition and enumeration of myeloblasts and ring sideroblasts. *Haematologica*. 2008;93:1712–1717.
5. Goasguen JE, Bennett JM, Bain BJ, et al. Morphological evaluation of monocytes and their precursors. *Haematologica*. 2009;94:994–997.
6. Bennet JM, Pryor J, Lughlin TS, et al. Is the association of “cup-like” nuclei with mutation of the NOP1 gene in acute myeloid leukemia clinically useful? *Am J Clin Pathol*. 2010;134:648–652.
7. McCormick SR, McCormick MJ, Grutkoski PS, et al. FLT3 mutations at diagnosis and relapse in acute myeloid leukemia. Cytogenetic and pathologic correlations, including cuplike blast morphology. *Arch Pathol Lab Med*. 2010;134:1143–1151.
8. Cheson BD, Cassileth PA, Head DR, et al. Report of the National Cancer Institute sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol*. 1990;8:813–819.
9. Goasguen JE, Bennett JM. Classification of acute myeloid leukemia. *Clin Lab Med*. 1990;10:661–681.
10. Bennett JM, Catovsky D, Daniel MT. Proposal for the recognition of minimally differentiated acute myeloid leukemia (AML-M0). *Br J Haematol*. 1991;78:325–329.
11. Neame PH, Soamboonsrup P, Browman GP, et al. Classifying acute leukemia by immunophenotyping. A combined FAB-immunologic classification of AML. *Blood*. 1986;68:1355–1362.
12. Lee EJ, Pollack A, Leavitt RD, et al. Minimally differentiated acute nonlymphocytic leukemia. A distinct entity. *Blood*. 1987;70:1400–1406.
13. Parreira A, Pombo de Oliverira MS, Matutes E, et al. Terminal deoxynucleotidyl transferase positive acute myeloid leukemia. An association with immature myeloblastic leukemia. *Br J Haematol*. 1988;69:219–224.
14. Brunning RD, Matutes E, Harris NL, et al. Acute myeloid leukaemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:75–107.
15. Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292–2302.
16. Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
17. Arber DA, Brunning RD, Orazi A, et al. Acute myeloid leukaemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:130–139.
18. Vardiman JW, Thiele J, Arber DA, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood*. 2009;114:937–951.
19. Li CY, Yam LT, Sun T. *Modern Modalities for the Diagnosis of Hematologic Neoplasms*. New York, NY: Igaku-Shoin; 1996:7–19.
20. Smith M, Barnett M, Bassan R, et al. Adult acute myeloid leukaemia. *Crit Rev Oncol Hematol*. 2004;50:197–222.
21. Bendix-Hansen K, Nielsen HK. Myeloperoxidase-deficient polymorphonuclear leukocytes. 2. Longitudinal study in acute myeloid leukemia, untreated, in remission and in relapse. *Scand J Haematol*. 1983;31:5–8.
22. Bennett JM, Begg CB. ECOG study of cytochemistry of acute myeloid leukemia by correlation of subtypes with response and survival. *Cancer Res*. 1981;41:4833–4837.
23. Hoyle CF, Gray RG, Wheatley K, et al. Prognostic importance of Sudan black positivity. A study of bone marrow slides from 1386 patients with de novo acute myeloid leukemia. *Br J Haematol*. 1991;70:398–407.

24. Gupta AM, Sapre RS, Shah AS, et al. Cytochemical and immunophenotypic heterogeneity in acute promyelocytic leukemia. *Acta Haematol.* 1989;81:5–8.
25. Scott CS, Cahill A, Morgan M, et al. Double esterase positive cells. *Br J Haematol.* 1984;58:762–794.
26. Jain NC, Cox C, Bennett JM. Auer rods in the acute myeloid leukemias. Frequency and methods of demonstration. *Hematol Oncol.* 1987;5:197–202.
27. Sun T. Comparison of immunohistochemistry and flow cytometry in immunophenotyping of hematologic neoplasms. *J Histotechnol.* 2004;27:101–109.
28. Sun T. Immunophenotyping of hematologic neoplasms by combined flow cytometry and immunohistochemistry. *J Clin Ligand Assay.* 2004;27:180–189.
29. Campana D. Determination of minimal residual disease in leukaemia patients. *Br J Haematol.* 2003;121:823–838.
30. Terstappen LWMM, Safford M, Konemann S, et al. Flow cytometric characterization of acute myeloid leukemia. 2. Phenotypic heterogeneity at diagnosis. *Leukemia.* 1992;6:70–80.
31. Krause DS, Fackler MJ, Civin CI, et al. CD34: structure, biology, and clinical utility. *Blood.* 1996;87:1–3.
32. Sawada K, Sato N, Notoya A, et al. Proliferation and differentiation of myelodysplastic CD34+ cells. Phenotypic subpopulations of marrow CD34+ cells. *Blood.* 1995;85:194–202.
33. Robertson MJ, Ritz J. Prognostic significance of the surface antigens expressed by leukemic cells. *Leuk Lymphoma.* 1994;13:15–22.
34. Traweek ST. Immunophenotypic analysis of acute leukemia. *Am J Clin Pathol.* 1993;99:504–512.
35. Dunphy CH. Comprehensive review of adult acute myelogenous leukemia. Cytomorphological, enzyme cytochemical, flow cytometric immunophenotypic, and cytogenetic findings. *J Clin Lab Anal.* 1999;13:19–26.
36. Hans CP, Finn WG, Singleton TP, et al. Usefulness of anti-CD117 in the flow cytometric analysis of acute leukemia. *Am J Clin Pathol.* 2002;117:301–305.
37. Rizzatti EG, Garcia AB, Portieres FL, et al. Expression of CD117 and CD11b in bone marrow can differentiate acute promyelocytic leukemia from recovering myeloid proliferations. *Am J Clin Pathol.* 2002;118:31–37.
38. Zimpfer A, Went P, Tzankov A, et al. Rare expression of KIT (CD117) in lymphomas: a tissue microarray study of 1166 cases. *Histopathology.* 2004;45:398–404.
39. Kita K, Miwa H, Nakase K, et al. Clinical importance of CD7 expression in acute myelocytic leukemia. *Blood.* 1993;81:2399–2405.
40. Del Poeta G, Stasi R, Venditti A, et al. Clinical importance of CD7 expression in acute myeloid leukemia. *Blood.* 1993;82:2929–2930.
41. Andriaansen HJ, te Broekhorst PAW, Hagemeijer AM, et al. Acute myeloid leukemia M4 with bone marrow eosinophilia (M4Eo) and inv(16)(p13q22) exhibits a specific immunophenotype with CD2 expression. *Am J Clin Pathol.* 2003;120:236–245.
42. Ball ED, Davis RB, Greffin JD, et al. Prognostic value of lymphocyte surface markers in acute myeloid leukemia. *Blood.* 1991;77:2242–2250.
43. Solary E, Casasnovas RO, Campos L, et al. Surface markers in adult acute myeloblastic leukemia. Correlation of CD19+, CD34+, and CD14+/DR– phenotypes with shorter survival. *Leukemia.* 1992;6:393–399.
44. Khalidi HS, Medeiro J, Chang KL, et al. The immunophenotype of adult acute myeloid leukemia. High frequency of lymphoid antigen expression and comparison of immunophenotype, French-American-British classification, and karyotypic abnormalities. *Am J Clin Pathol.* 1998;109:211–220.
45. Pui CH, Campana D, Crist WM. Toward a clinically useful classification of the acute leukemias. *Leukemia.* 1995;9:2154–2157.
46. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias. *Leukemia.* 1995;9:1783–1786.
47. Mirrour J, Zipf TFD, Pui CH, et al. Acute mixed lineage leukemia. Clinicopathologic correlations and prognostic significance. *Blood.* 1985;66:1115–1123.
48. Cross AH, Goorha RM, Nuss R, et al. Acute myeloid leukemia with T-lymphoid features: a distinct biologic and clinical entity. *Blood.* 1988;72:579–587.
49. Borowitz MJ, Bene MC, Harris NL, et al. Acute leukaemias of ambiguous lineage. In Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2008:150–155.
50. Drexler HG, Sperling C, Ludwig WD. Terminal deoxynucleotidyl transferase (TdT) expression in acute myeloid leukemia. *Leukemia.* 1993;7:1142–1150.
51. Lee EJ, Yang J, Leavitt RD, et al. The significance of CD34 and TdT determinations in patients with untreated de novo acute myeloid leukemia. *Leukemia.* 1992;6:1203–1209.
52. Calwell CW, Patterson WP, Toalson BD, et al. Surface and cytoplasmic expression of CD45 antigen isoforms in normal and malignant myeloid cell differentiation. *Am J Clin Pathol.* 1991;95:180–187.
53. Scott AA, Head DR, Kopecky KU, et al. HLA-DR–, CD33+, CD56+, CD16– myeloid/natural killer cell acute leukemia. A previously unrecognized form of acute leukemia potentially misdiagnosed as French-American-British acute myeloid leukemia-M3. *Blood.* 1994;84:244–255.
54. Arber DA, Jenkins KA. Paraffin section immunophenotyping of acute leukemias in bone marrow specimens. *Am J Clin Pathol.* 1996;106:462–468.
55. Mrozek K, Heinonen K, de la Chapelle A, et al. Clinical significance of cytogenetics in acute myeloid leukemia. *Semin Oncol.* 1997;24:17–31.
56. Caligiuri MA, Strout MP, Gilliland DG. Molecular biology of acute myeloid leukemia. *Semin Oncol.* 1997;24:32–44.
57. Head DR. Revised classification of acute myeloid leukemia. *Leukemia.* 1996;10:1826–1831.
58. Betz B, Hess JL. Acute myeloid leukemia diagnosis in the 21st century. *Arch Pathol Lab Med.* 2001;134:1427–1433.
59. Valk PJ, Delwel R, Löwenberg B. Gene expression profiling in acute myeloid leukemia. *Curr Opin Hematol.* 2005;12:76–81.
60. Frankfurt O, Licht JD, Tallman MS. Molecular characterization of acute myeloid leukemia and its impact on treatment. *Curr Opin Oncol.* 2007;19:635–649.
61. Bagg A. Clinical applications of molecular genetic testing in hematologic malignancies: advantages and limitations. *Hum Pathol.* 2003;34:352–358.
62. Bacher U, Schnittger S, Haferlach T. Molecular genetics in acute myeloid leukemia. *Curr Opin Oncol.* 2010;22:646–655.
63. Gilliland DG, Griffin JD. The role of FLT3 in hematopoiesis and leukemia. *Blood.* 2002;100:1532–1542.
64. Langabeer SE, Walker H, Gale RE, et al. Frequency of CBF beta/MYH11 fusion transcripts in patients entered into the U.K. MRC AML trials. The MRC Adult Leukaemia Working Party. *Br J Haematol.* 1997;96:736–739.
65. Langabeer SE, Walker H, Rogers JR, et al. Incidence of AML1/ETO fusion transcripts in patients entered into the MRC AML trials. MRC Adult Leukaemia Working Party. *Br J Haematol.* 1997;99:925–928.

66. Grimwade D, Biondi A, Mozziconacci MJ, et al. Characterization of acute promyelocytic leukemia cases lacking the classic t(15;17): results of the European Working Party. Groupe Francais de Cytogenetique Hematologique. Groupe de Francais d'Hematologie Cellulaire, U.K. Cancer Cytogenetics Group and BIOMED 1 European Community-Concerted Action "Molecular Cytogenetic Diagnosis in Haematological Malignancies." *Blood*. 2000;96:1207–1308.
67. Armstrong SA, Staunton JE, Silverman LB, et al. MLL translocations specify a distinct gene expression profile that distinguishes a unique leukemia. *Nat Genet*. 2002;30:41–47.
68. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science*. 1999;286:531–537.
69. Kohlmann A, Schoch C, Schnittger S, et al. Molecular characterization of acute leukemias by use of microarray technology. *Genes Chromosomes Cancer*. 2003;37:396–405.
70. Schoch C, Hohlmann A, Schnittger S, et al. Acute myeloid leukemias with reciprocal rearrangements can be distinguished by specific gene expression profiles. *Proc Natl Acad Sci U S A*. 2002;99:10008–10013.
71. Rose NE, Mahfouz R, Onciu M, et al. Gene expression profiling of pediatric acute myelogenous leukemia. *Blood*. 2004;104:3670–3687.
72. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med*. 1999;341:1051–1062.
73. Ravindranath Y. Recent advances in pediatric acute lymphoblastic and myeloid leukemia. *Curr Opin Oncol*. 2003;15:23–35.
74. Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). *Blood*. 2002;100:4325–4336.
75. Grimwade D, Vyas P, Freeman S. Assessment of minimal residual disease in acute myeloid leukemia. *Curr Opin Oncol*. 2010;22:656–663.

CASE 9

Acute Myeloblastic Leukemia with Maturation

CASE HISTORY

A 52-year-old man had the chief complaint of shortness of breath and fatigue. He was admitted to another hospital and was found to have anemia. A gastrointestinal workup showed nothing remarkable. A bone marrow biopsy was performed; the diagnosis was myelodysplastic syndrome: refractory anemia with excess blasts. He was treated with Gleevac and prednisone to no avail. The patient had become transfusion dependent.

When the patient was transferred to our hospital 1 year later, physical examination showed no hepatosplenomegaly or lymphadenopathy. His total leukocyte count was 56,000/mL with 44% blasts and 38% myeloid cells of various developmental stages, but only 1% monocytes were demonstrated. Bone marrow examination revealed 68% myeloblasts and 23% other myeloid cells. The monocytes were <1%. No basophils were identified.

FLOW CYTOMETRIC FINDINGS

Bone marrow: Myeloid markers: myeloperoxidase (MPO) 20%, CD13-CD33 97%, CD14 0%, HLA-DR 85%. T-cell marker: CD7 0%. Immature cell markers: CD34 51%, CD117 74% (Fig. 6.9.1).

MOLECULAR GENETICS

The karyotype was 46, XY, t(6;9)(p23;q34). A DEK-NUP214(CAN) fusion gene was detected by reverse transcriptase–polymerase chain reaction.

DISCUSSION

The definition of acute myeloblastic leukemia (AML) with maturation or AML M2 by French-American-British (FAB) classification includes the following criteria (1–7): (a) The myeloblast count should be $\geq 30\%$ in the bone marrow or peripheral blood. The World Health Organization (WHO) system changes the threshold to 20% based on clinical trials. (b) The mature myeloid population from the segmented neutrophil to promyelocyte should be $>10\%$ in the bone marrow to distinguish AML-M2 from AML without maturation (AML-M1). (c) Monocytic components should be $<20\%$ in the bone marrow and $<5 \times 10^9/L$ in the peripheral blood to exclude acute myelomonocytic leukemia (AML-M4). Monoblasts are sometimes difficult to distinguish from myeloblasts. In those cases, cytochemical study is required to distinguish AML-M2 from AML-M4.

In addition, the blast count should include blast type 1 (no cytoplasmic granules), blast type 2 (<20 cytoplasmic granules), and blast type 3 (>20 cytoplasmic granules). However, when blast type 3 is $>10\%$, the leukemic case should be classified as AML with maturation, even though the mature granulocytes are $<10\%$ (1). Blast types 2 and 3 can be distinguished from promyelocytes by the centrally located nucleus, absence of a prominent Golgi zone, and presence of a fine chromatin pattern. AML with maturation is the most common subtype of AML, accounting for 25% to 45% of AML cases (4,8).

Morphology

In addition to what was described in the definition, this subtype frequently shows dysplastic changes in myeloid

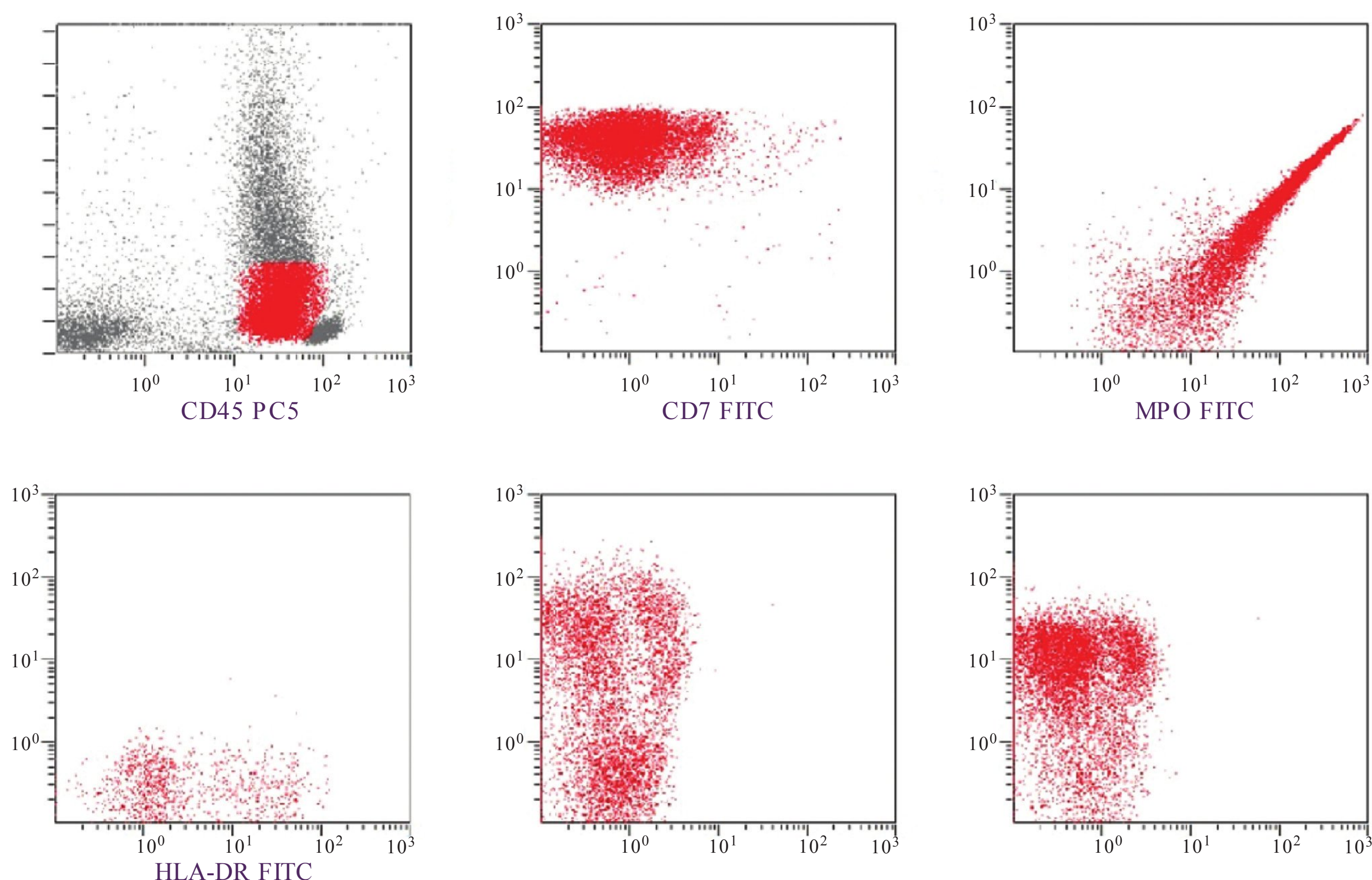


FIGURE 6.9.1 Flow cytometric analysis of bone marrow shows a tight cluster of leukemic cells in the CD45/side-scatter gating and a large population of maturing granulocytes with high side scatter. The population of leukemic cells is characterized by myeloid markers CD13-CD33, MPO, and by stem cell markers CD34 and CD117. HLA-DR is weakly positive. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

cells, including abnormal nuclear segmentation and hypogranulation (4,7). Eosinophilia may also be present in the bone marrow, but it does not show the cytologic or cytochemical abnormalities characteristic of acute myelomonocytic leukemia with eosinophilia (4,7).

In the current case, the myeloblast count was 44% in the peripheral blood (Fig. 6.9.2) and 68.7% in the bone marrow (Figs. 6.9.3 and 6.9.4). No monoblasts were demonstrated in the bone marrow, and <1% of monocytes were present. Therefore, morphologically, it fulfills the definition of AML-M2.

AML-M2 cases with $t(6;9)(p23;q34)$, such as our case, are frequently associated with myelodysplastic syndrome and basophilia in the bone marrow (2,9). The current case had a history of myelodysplastic syndrome, but basophilia was not demonstrated in the bone marrow. According to the 2008 WHO classification, this case should be categorized as AML with $t(6;9)(p23;q34)$;DEK-NUP214 (9).

Cytochemistry

The FAB system requires three cytochemical stains as the basis for AML classification. In M2, the MPO should be positive for >3% of blasts. The specific esterase or chloroacetate esterase (CAE) should be positive, and the non-specific esterase or α -naphthyl butyrate esterase (NBE)

should be negative for the blasts (Fig. 6.9.5). However, in some M2 cases, NBE was negative and yet focal staining of α -naphthyl acetate esterase (another monocyte marker) was demonstrated in most myeloblasts (10).

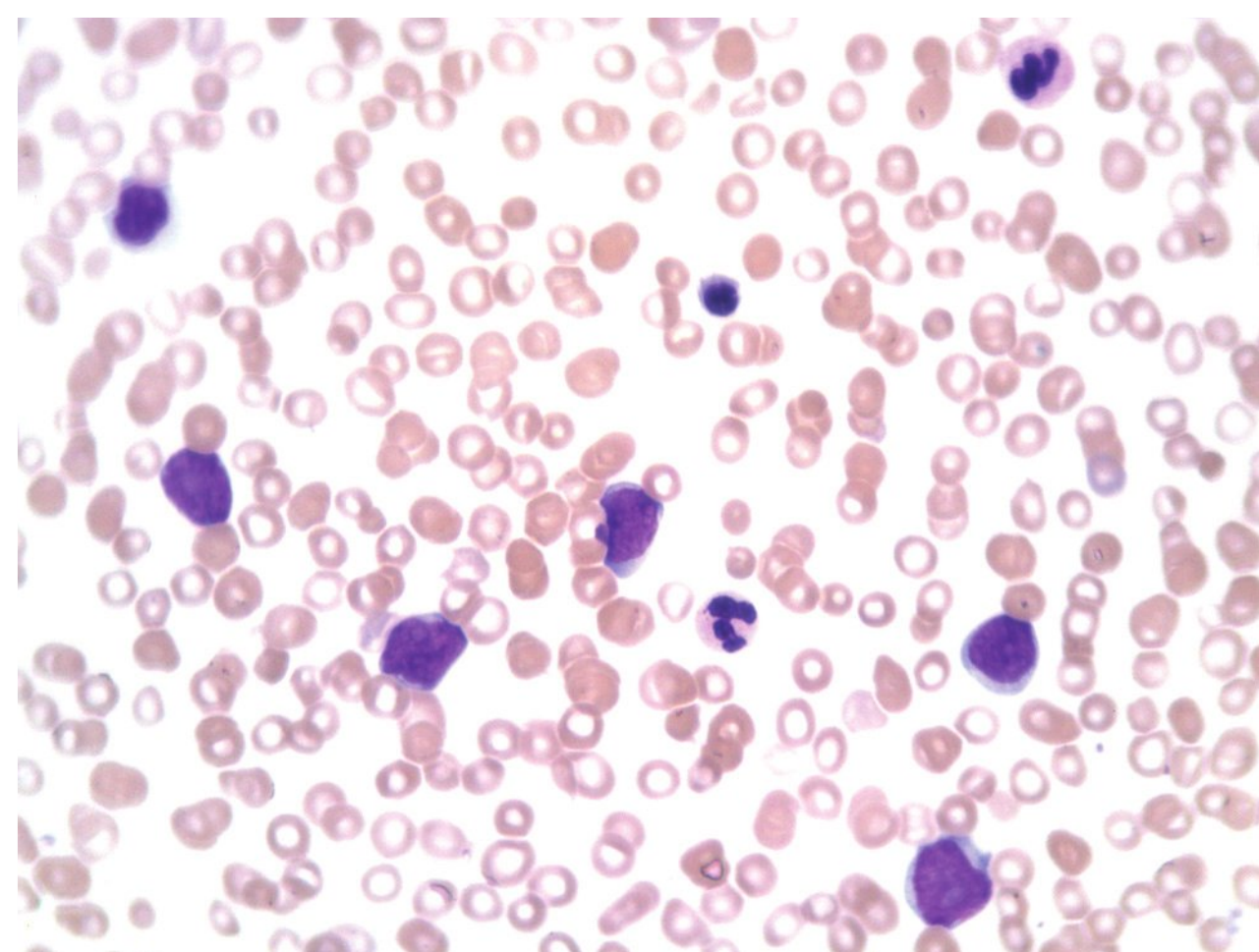


FIGURE 6.9.2 Peripheral blood smear shows myeloblasts and a few mature granulocytes. Wright–Giemsa, 40× magnification.

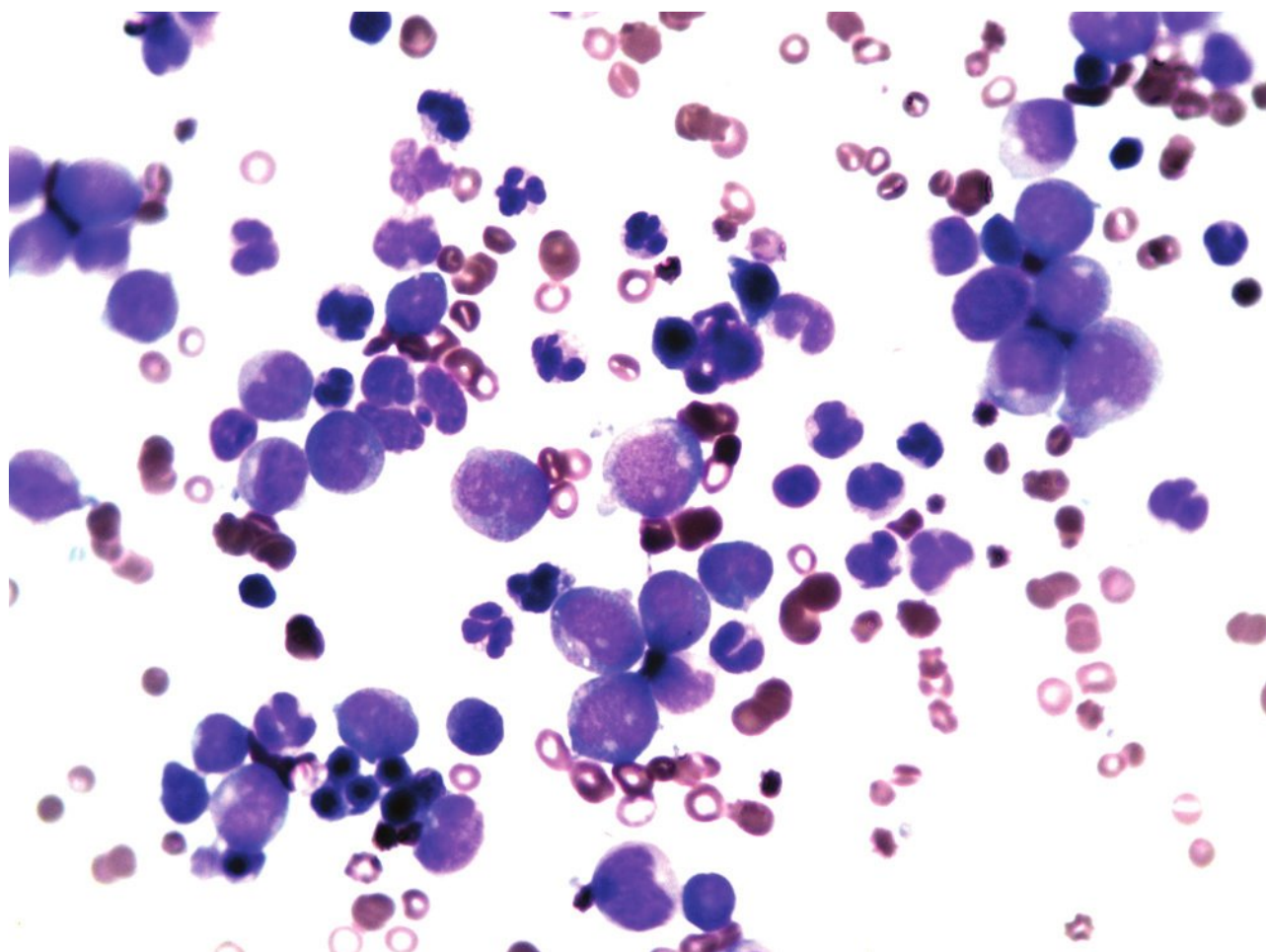


FIGURE 6.9.3 Bone marrow aspirate reveals a high percentage of myeloblasts intermingled with mature granulocytes. Wright–Giemsa, 60× magnification.

A British group proposed using >50% of blasts with Sudan black B positivity as a cutoff to distinguish M2 from M1; the latter usually had <50% Sudan black B–positive blasts in the bone marrow (11). MPO is frequently stronger in M2 than in M1. When very strong MPO activity and abundant Auer rods are present in neutrophils and eosinophils in an M2 case, t(8;21) should be suspected (12,13) (see Case 4).

Immunophenotype

The FAB system uses morphology and cytochemical staining to classify AML. However, because immunophenotyping is now commonly applied to AML classification, cytochemical staining is no longer essential. For instance, in cases of minimally differentiated AML in which MPO stain is negative, immunophenotyping by

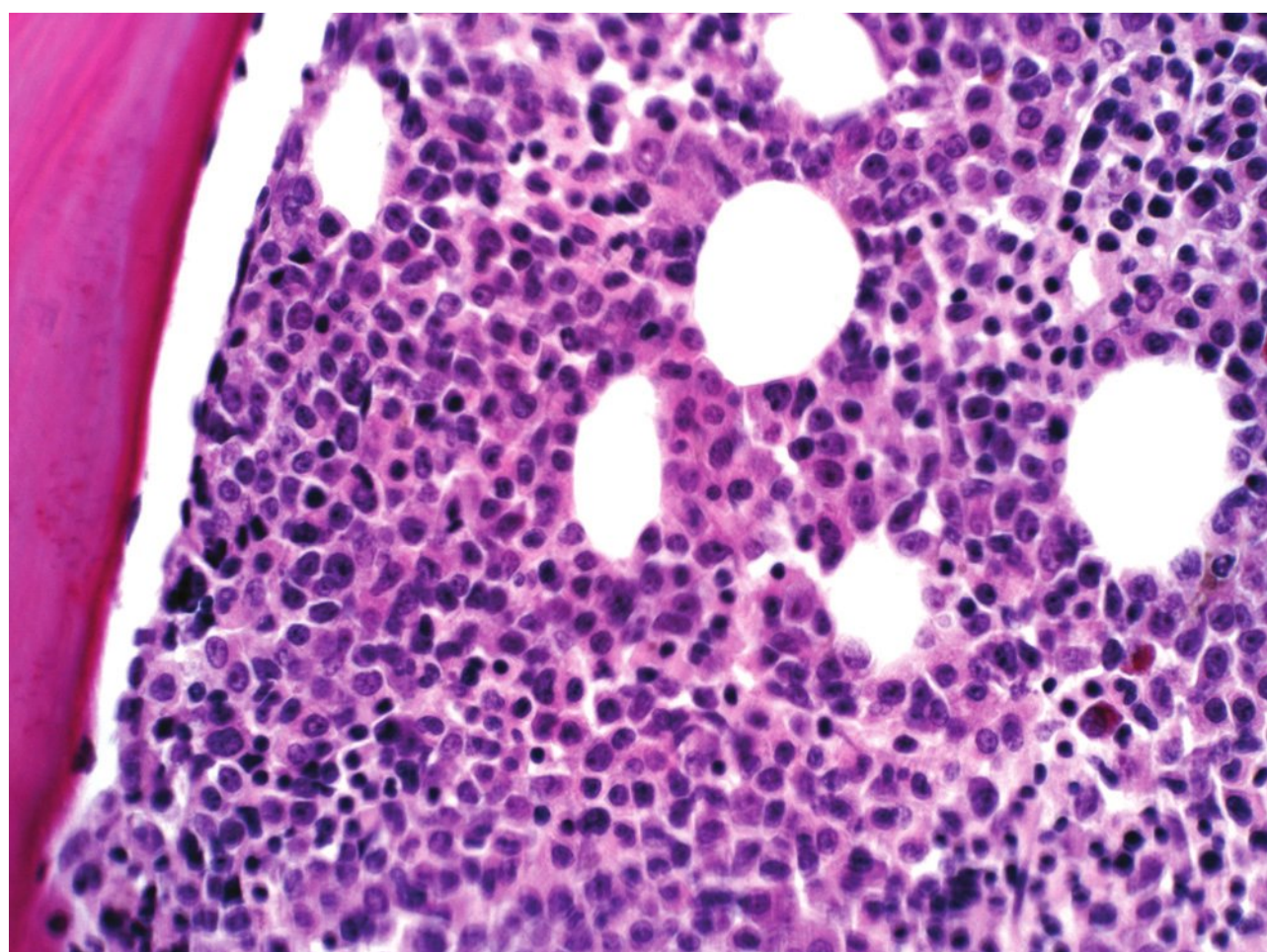


FIGURE 6.9.4 Bone marrow core biopsy demonstrates hypercellularity with mature and immature myeloid cells. No erythroid elements are demonstrated. Hematoxylin and eosin, 40× magnification.

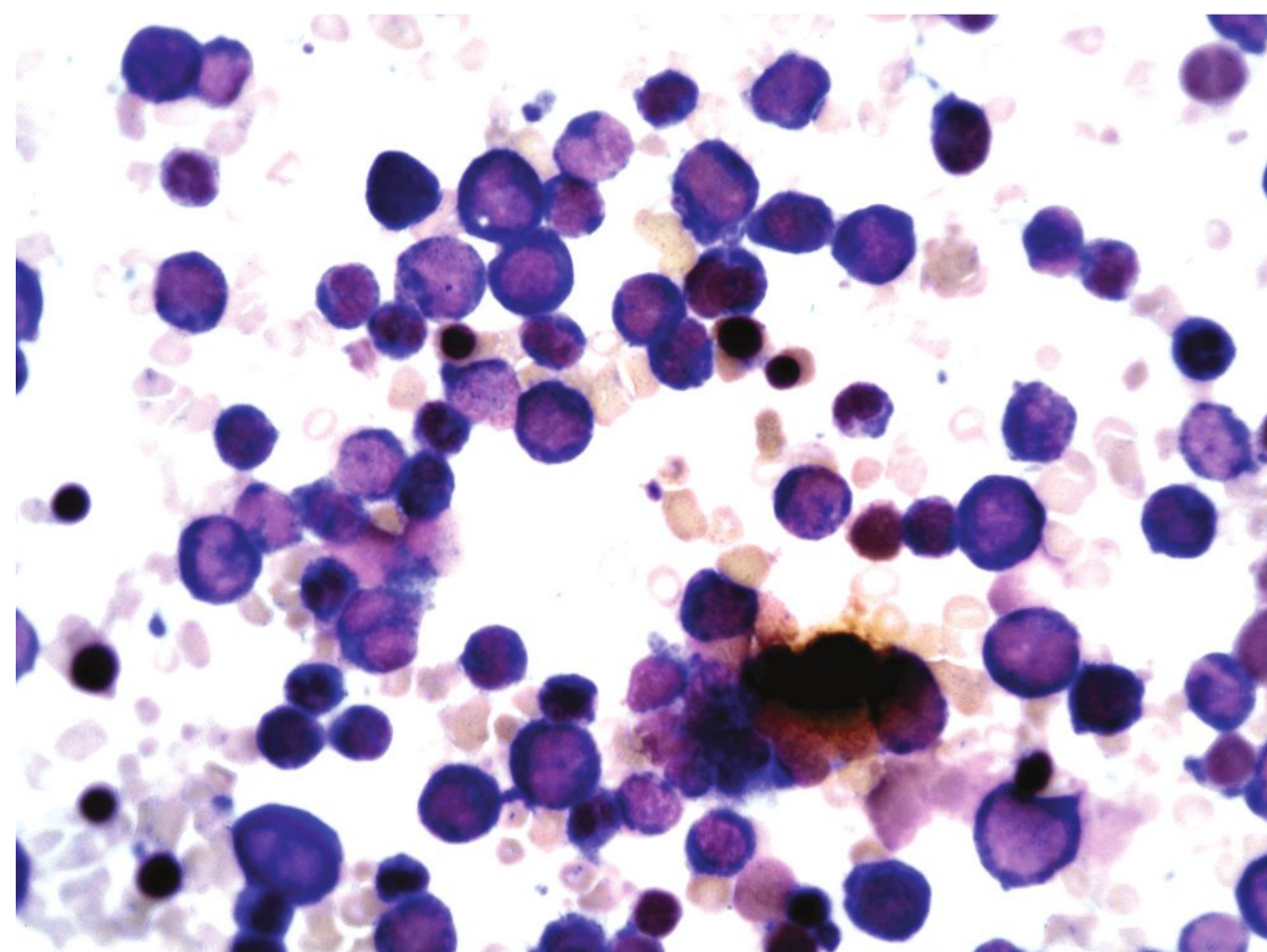


FIGURE 6.9.5 Bone marrow aspirate stained with combined esterases. All myeloblasts and different developmental stages of myeloid cells stained with CAE (blue). Only one monocyte is stained by a-NBE (brown). Combined esterase stain, 60× magnification.

flow cytometry is critical to distinguish it from acute lymphoblastic leukemia.

A monoclonal antibody panel including CD13, CD14, CD15, CD33, CD64, and HLA-DR is sufficient to make a preliminary classification of AML subtypes (14). Whereas CD13 and CD33 are screening markers for AML, others help to differentiate the subtypes. CD14 and CD64 are present in monocytic subtypes (M4 and M5). HLA-DR is low or absent in acute promyelocytic leukemia. CD15 is supposed to be negative in M1 and can help to distinguish other subtypes, but it may be present in some M1 cases and is thus not very specific.

The malignant nature of the myeloid population is identified by two markers, CD34 and CD117. CD34 is a hematopoietic progenitor antigen; therefore, a high percentage of CD34-positive cells is suggestive of leukemia or myelodysplasia (15,16). CD34 is preferentially present in the most immature phenotypes (M1, M2, and M5a) (17). Recently, CD117, a stem cell factor receptor also known as c-kit, has been used to supplement CD34 in identifying myeloblasts. CD117 is negative in cases of acute lymphoblastic leukemia, but it is positive in cases of AML including acute promyelocytic leukemia (M3) (18).

AML may also express lymphoid markers, such as CD2, CD7, CD10, CD19, and CD20 (6). The demonstration of a single lymphoid marker in a myeloid population does not indicate a biphenotypic leukemia; rather this aberrant immunophenotype supports the diagnosis of malignancy. CD7 is the most commonly expressed marker, found in 19% to 32% of AML cases depending on the subtypes (6,19). Therefore, CD7 has been included in the diagnostic panel for AML in many laboratories.

Immunohistochemistry may demonstrate myeloid markers, such as MPO, lysozyme, and CAE (Leder stain). CD34 (Fig. 6.9.6) and CD117 (Fig. 6.9.7) may also be

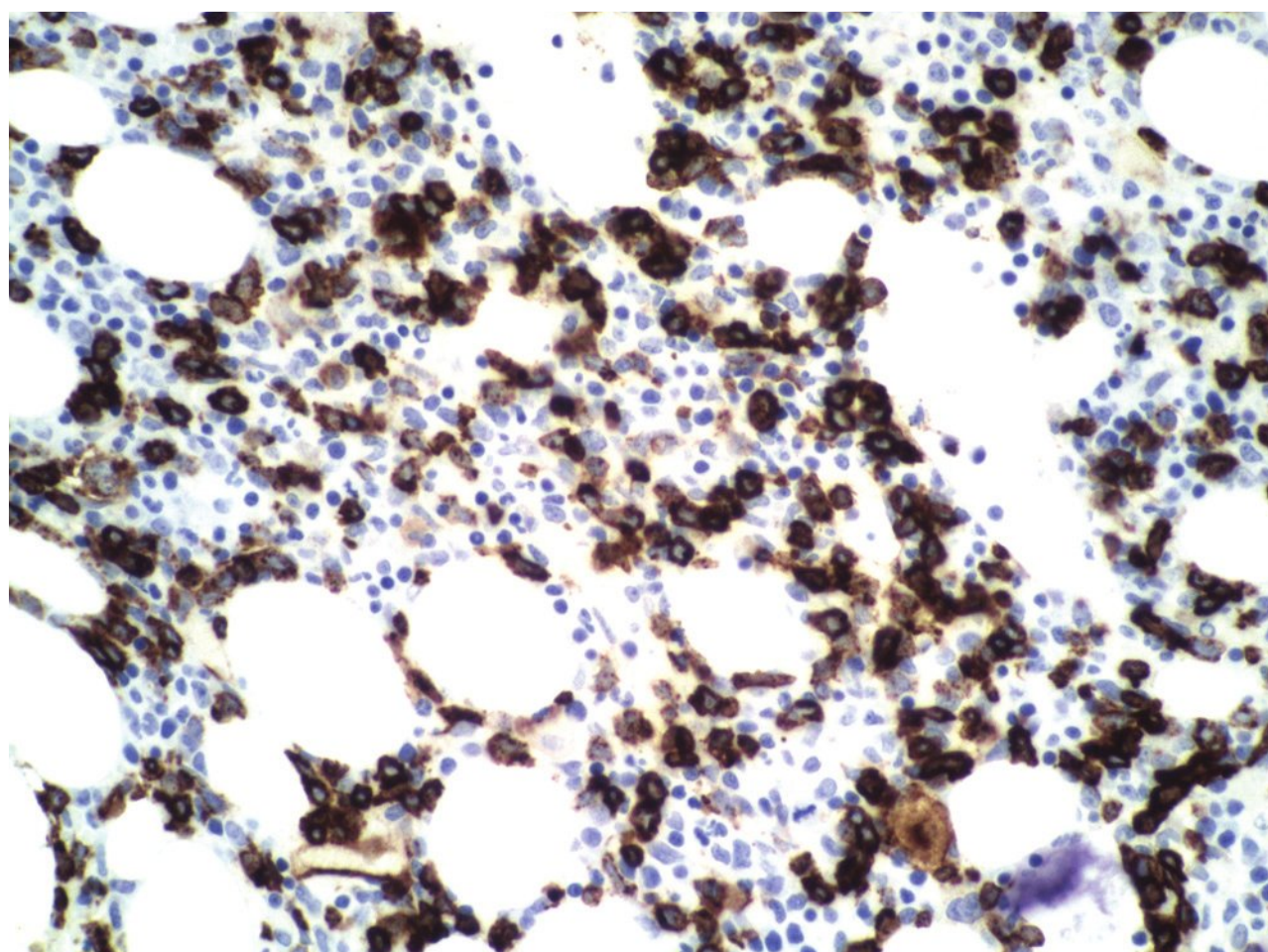


FIGURE 6.9.6 Bone marrow biopsy shows scattered CD34-positive myeloblasts. Immunoperoxidase, 40× magnification.

demonstrated by immunohistochemical stains, which, however, are usually less sensitive than flow cytometry. The salient features for laboratory diagnosis of AML with maturation are summarized in Table 6.9.1.

Molecular Genetics

The most frequent cytogenetic abnormality seen in about 46% of M2 cases is $t(8;21)(q22;q22)$ (20). This is classified as a separate subtype in the WHO system and is described in Case 4.

Another group of M2 is associated with basophilia in the bone marrow. At least two abnormal karyotypes have been found in this group of M2: $t/del(12)(p11-13)$ and $t(6;9)(p23;q34)$ (2). In these cases, the blasts are agranular, but other cells show evidence of maturation toward basophils (21). Basophilic granules can also be detected in a few blasts by electron microscopy. The

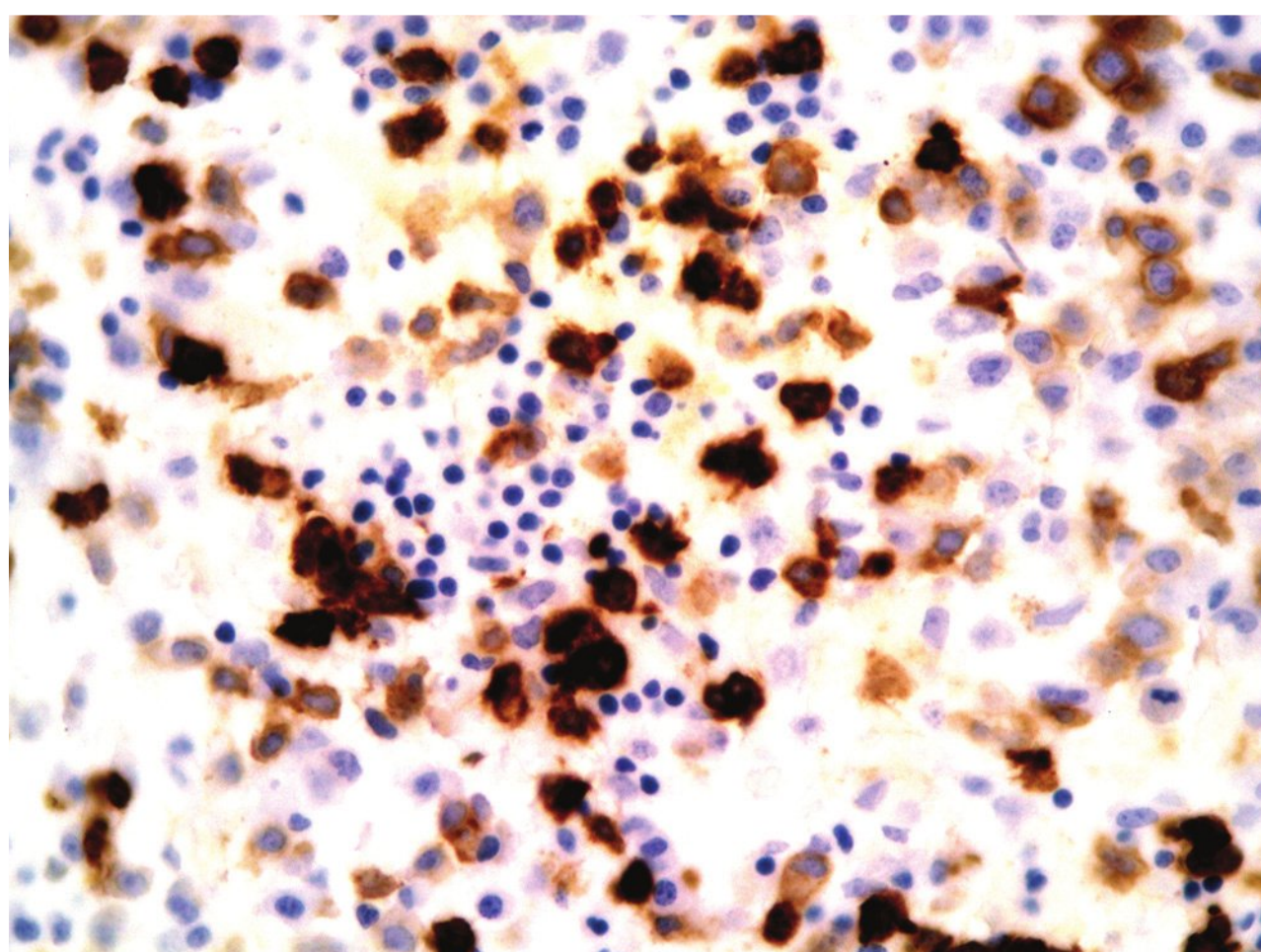


FIGURE 6.9.7 Bone marrow biopsy shows scattered CD117-positive myeloblasts. Immunoperoxidase, 40× magnification.

TABLE 6.9.1

Salient Features for Laboratory Diagnosis of AML with Maturation

1. 20–90% of myeloblasts present in bone marrow or blood
2. <20% monocytic components in the bone marrow
3. $<5 \times 10^9/L$ monocytic components in the peripheral blood
4. Cytochemical stain for blasts: positive for MPO and CAE but negative for α -naphthyl butyrate esterase
5. Monoclonal antibody panel: positive for CD13, CD15, CD33, HLA-DR; negative for CD14 and CD64
6. CD34, CD117, and CD7 can be positive to support the malignant nature.
7. Immunohistochemistry: positive for lysozyme, MPO, CAE, CD34, or CD117

$t(6;9)(p23;q34)$ results in the formation of a chimeric fusion gene: DEK-NUP214 (formerly DEK-CAN) (4,9). The fusion of the nuclear phosphoprotein DEK and the nucleoporin NUP214 produces a fusion protein that acts as an aberrant transcription factor as well as altering nuclear transport by binding to soluble transport factors (9). Another study shows that the expression of DEK-NUP214 correlates to the phosphorylation of the translation initiation protein, EIF4E (22). As a result, translation activity is elevated and a substantial increase in global protein synthesis is found in DEK-NUP214-expressing cells. This is considered the possible mechanism in the pathogenesis of this type of AML.

FLT3-ITD mutations are very common in AML with this karyotype (9). A recent study showed that 69% of pediatric patients and 73% of adult patient had FLT-ITD mutations (23). On the other hand, there is a low incidence of secondary aberrations in $t(6;9)$ positive cases (23).

There are several rare abnormal karyotypes reported in M2 cases. These include $inv(3)(q21;q26)$, $t(3;3)(q21;q26)$, $t(7;11)(p15;p15)$, $t(6;21)(p11;q22)$, $+11(2.23)$, and double minute chromosomes (2,24,25). A rare karyotype of $t(8;16)(p21;p13)$ is associated with hemophagocytosis, particularly erythrophagocytosis (26).

Clinical Manifestations

AML with maturation occurs in all age groups with 40% seen in patients older than 60 years and 25% in patients younger than 25 years (4). A recent study of 69 cases found that the patients' age ranged from 2 to 66 years with a median of 23 years (23). The clinical symptoms are not different from other subtypes of AML. The major mechanisms are failure of the leukemic cells to mature and inhibition of normal hematopoiesis. As a result, the patients may have anemia, neutropenia, and/or thrombocytopenia. This subtype of AML usually responds well to chemotherapy, but the prognosis is frequently associated with the abnormal karyotype of the

leukemic cells. For instance, the overall survival of adult patients with t(6;9) is 9% to 12% (23). The 5-year survival estimate was 28% for pediatric patients and 9% for adult patients in one study (23).

REFERENCES

1. Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med.* 1985;103:620–624.
2. Second MIC Cooperative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukemias. *Br J Haematol.* 1988;68:487–494.
3. Goasguer JE, Bennett JM. Classification of acute myeloid leukemia. *Clin Lab Med.* 1990;10:661–681.
4. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia not otherwise categorised. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:93–94.
5. Brunning R. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
6. Smith M, Barnett M, Bassan R, et al. Adult acute myeloid leukaemia. *Crit Rev Oncol Hematol.* 2004;50:197–222.
7. Arber DA, Brunning RD, Orazi A, et al. Acute myeloid leukaemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed. Lyon, France: IARC Press; 2008:131–139.
8. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med.* 1999;341:1051–1062.
9. Arber DA, Brunning RD, Le Beau MM, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed. Lyon, France: IARC Press; 2008:110–123.
10. Elghetany MT, MacCallum JM, Davey FR. The use of cytochemical procedures in the diagnosis and management of acute and chronic myeloid leukemia. *Clin Lab Med.* 1990;10:707–720.
11. Hoyle CR, Gray RG, Wheatley K, et al. Prognostic importance of Sudan black positivity: a study of bone marrow slides from 1386 patients with de novo acute myeloid leukemia. *Br J Haematol.* 1991;79:398–407.
12. Hayhoe FGJ. Cytochemistry of acute leukemias. *Histochem J.* 1984;16:1051–1059.
13. Yunis JJ, Lobell M, Arnesen MA, et al. Refined chromosome study helps define prognostic subgroups in most patients with primary myelodysplastic syndrome and acute myelogenous leukemia. *Br J Haematol.* 1988;68:189–194.
14. Neame PB, Soamboonsrup P, Browman GP, et al. Classifying acute leukemia by immunophenotyping: a combined FAB immunologic classification of AML. *Blood.* 1986;68:1355–1362.
15. Krause DS, Fackler MJ, Givin CI, et al. CD34: structure, biology, and clinical utility. *Blood.* 1996;87:1–13.
16. Sawada K, Sato N, Notoya A, et al. Proliferation and differentiation of myelodysplastic CD34+ cells: phenotypic subpopulations of marrow CD34+ cells. *Blood.* 1995;85:194–202.
17. Robertson MJ, Ritz J. Prognostic significance of the surface antigens expressed by leukemic cells. *Leuk Lymphoma.* 1994;13:15–22.
18. Hans CP, Finn WG, Singleton TP, et al. Usefulness of anti-CD117 in the flow cytometric analysis of acute leukemia. *Am J Clin Pathol.* 2002;117:301–305.
19. Kita K, Miwa H, Nakase K, et al. Clinical importance of CD7 expression in acute myelocytic leukemia. *Blood.* 1993;81:2399–2405.
20. Nucifora G, Rowley JD. The AML and ETO genes in acute myeloid leukemia with a t(8;21). *Leuk Lymphoma.* 1994;14:353–362.
21. Daniel MT, Bernheim A, Flandrin G, et al. Leucemic Aigue Myeloblastique (M2) avec atteinte de la lignee basophile et anomalies du bras court du chromosome 12(12p-). *C R Acad Sci Paris.* 1985;301:299.
22. Ageberg M, Drott K, Olofsson T, et al. Identification of a novel and myeloid specific role of the leukemia-associated fusion protein DEK-NUP214 leading to increased protein synthesis. *Genes Chrom Cancer.* 2008;47:276–287.
23. Slovak ML, Gundacker H, Bloomfield CD, et al. A retrospective study of 69 patients with t(6;9)(p23;q34) AML emphasizes the need for a prospective, multicenter initiative for rare ‘poor prognosis’ myeloid malignancies. *Leukemia.* 2006;20:1295–1297.
24. Fujimura T, Ohyashiki K, Ohyaskiki JH, et al. Two additional cases of acute myeloid leukemia with t(7;11)(p15;p15) having low neutrophil alkaline phosphatase scores. *Cancer Genet Cytogenet.* 1993;68:143–146.
25. Thomas L, Stamberg J, Gojo I, et al. Double minute chromosomes in monoblastic (M5) and myeloblastic (M2) acute myeloid leukemia: two case reports and a review of the literature. *Am J Hematol.* 2004;77:55–61.
26. Mrozek K, Heinonen K, de la Chapelle A, et al. Clinical significance of cytogenetics in acute myeloid leukemia. *Semin Oncol.* 1997;24:17–31.

CASE 10

Acute Myelomonocytic Leukemia

CASE HISTORY

A 60-year-old man presented to the emergency room because of a 1-day history of severe epistaxis. He first noticed small amount of bleeding from his nose a few days before, but it stopped spontaneously. The patient also complained of a 40-lb weight loss in the past several months. On admission, he was found to have a hematocrit of 18% as compared with his previous record of 38% a few months ago. His platelet count was 120,000/mL. His leukocyte count was 6,400/ μ L with 66% neutrophils, 18% lymphocytes, and 18% monocytes. After blood transfusion and intravenous fluids, the patient's condition was stabilized and his bleeding stopped. An ear, nose, and throat physician was consulted, but he did not find any visible nasal masses. Because of positive occult blood in the stool, a computed tomography scan of the abdomen, a colonoscopy, and an upper endoscopy were performed. A sigmoid colon polyp was found, but biopsy showed no malignancy. The patient was discharged 1 week after admission.

During the subsequent follow-up period, the patient's platelet count gradually returned to normal, but his hematocrit remained at low level (30%) and hemoglobin, 10 g/dL. Hypersegmented and hypogranular granulocytes, giant platelets, spherocytosis, and polychromasia were seen in the peripheral blood. The patient was considered to have myelodysplastic syndrome. However, his leukocyte count rapidly rose to 30,000/mL and the blasts gradually went up to 21% (Fig. 6.10.1). Monocytosis was also present. Bone

marrow examination showed 16% myeloblasts, 15% monoblasts and/or promonocytes, 26% monocytes, 2.5% promyelocytes, 6.7% myelocytes, 7.3% metamyelocytes, 12.3% bands, and 5.3% segmented granulocytes. Only 6% of erythroid series was demonstrated (Figs. 6.10.2 and 6.10.3). A diagnosis of acute myeloid leukemia (AML) was established approximately 2 months after first admission.

FLOW CYTOMETRIC FINDINGS

Bone marrow: Myelomonocytic markers: CD13-CD33 98%, CD13-CD33/CD7 40%, CD14 30%, HLA-DR 94%, CD34 89%, CD117 93% (Fig. 6.10.4).

CYTOCHEMICAL FINDINGS

In the bone marrow aspirate smear, myeloperoxidase (MPO) stain was positive in >5% of blasts. The chloroacetate esterase (CAE) and α -naphthyl butyrate esterase (NBE) stains demonstrated approximately the same percentages of myeloid and monocytoid cells.

DISCUSSION

Acute myelomonocytic leukemia (M4) accounts for 20% to 30% of AML cases (1–3). Therefore, it is as common as AML M2 in frequency. The diagnostic criterion as defined by

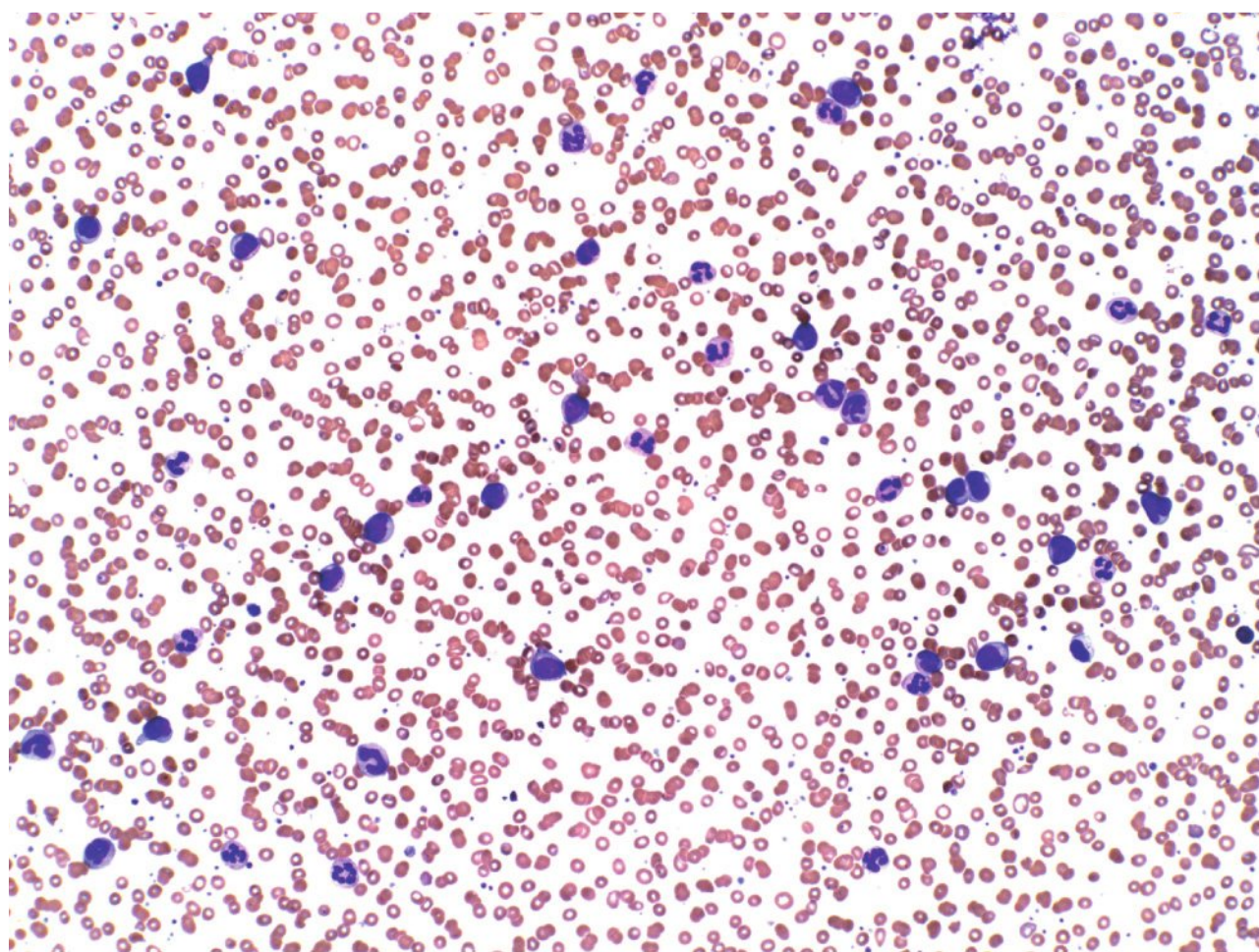


FIGURE 6.10.1 Peripheral blood smear shows leukocytosis consisting of mature and immature myelomonocytic cells. Wright-Giemsa, 20 $\times$ magnification.

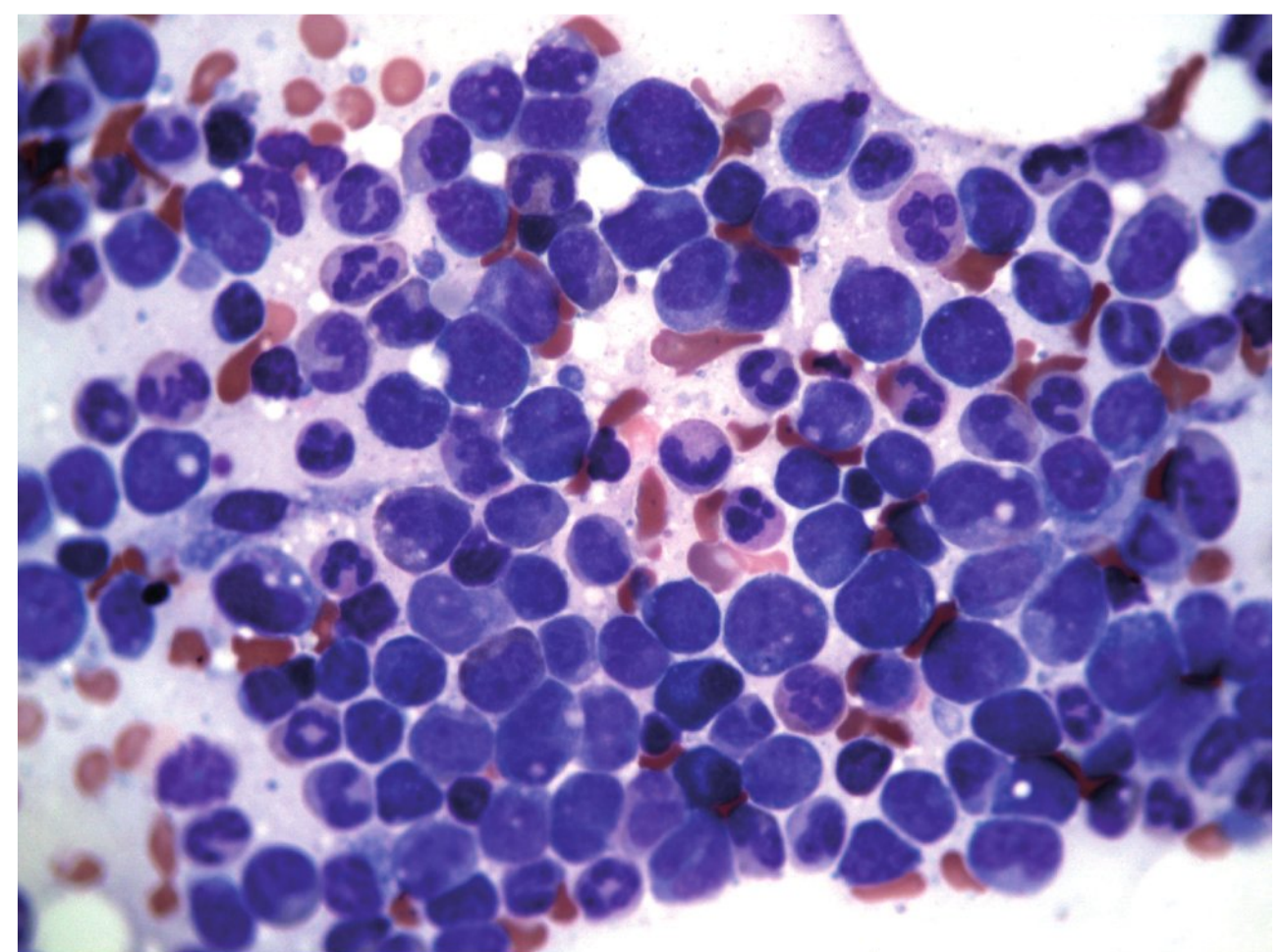


FIGURE 6.10.2 Bone marrow aspirate shows a packed marrow with mostly immature myeloid and monocytoid cells. Wright-Giemsa, 60 $\times$ magnification.

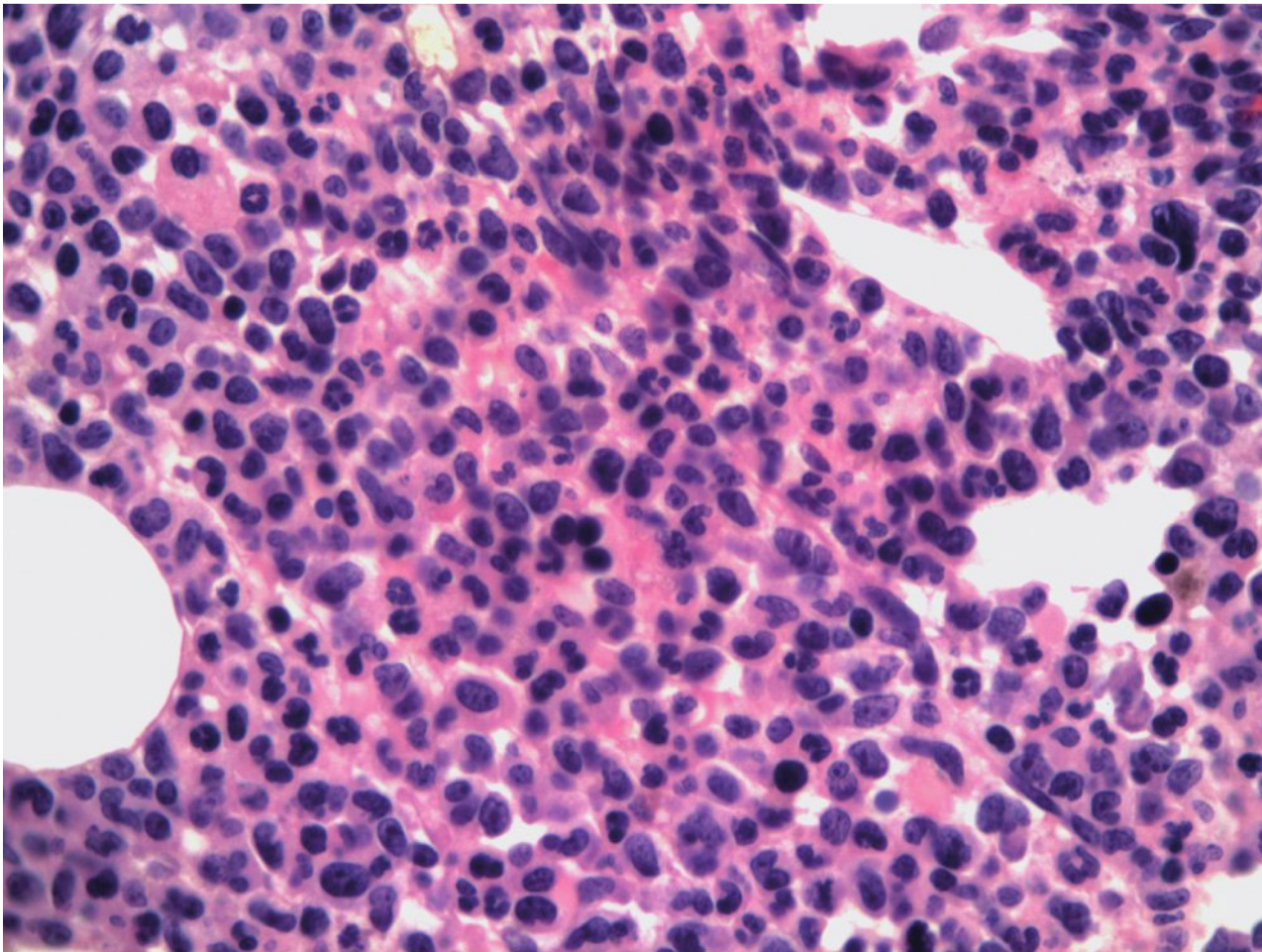


FIGURE 6.10.3 Bone marrow core biopsy reveals hypercellular bone marrow with myelomonocytic leukemic cells replacing the normal hematopoietic components. Hematoxylin and eosin, 20× magnification.

the French-American-British (FAB) group is the presence of 30% of blasts in the bone marrow, including type I and type II myeloblasts, monoblasts, and promonocytes (4). The World Health Organization classification, however, lowers the cutoff point of blasts in the bone marrow to 20% (2,5). In the differential count, both the granulocytic

and monocytic components should exceed 20%; below this threshold, the leukemia is classified as M5 or M2, respectively.

Cytochemical stains should be used to determine the percentages of myeloid and monocytoid cells. The monocytoid cells can be identified by nonspecific esterase, and the myeloid cells by specific esterase (see Case 8). The identification of monocytoid cells in the bone marrow is particularly difficult by morphology. However, nonspecific esterase can be weak or absent in monocytoid cells in some cases. If morphologic identification of monocytes is certain, absence of nonspecific esterase does not exclude the diagnosis of M4 (2).

When the percentage of monocytoid cells in bone marrow is <20%, the peripheral blood should have $>5 \times 10^9/L$ monocytes to meet the diagnostic criteria (4). When the monocyte count is below that level, a high lysozyme concentration can be used as evidence for a significant monocytosis, thus substantiating the diagnosis of M4 (4). The lysozyme concentrations should exceed three times the normal values in serum or urine to be considered significant. The only exception is the subtype of M2 with eosinophilia that may show a high lysozyme level because eosinophils also contain lysozyme (6).

In the current case, the first presentation in the preleukemic phase was epistaxis, which led to the discovery of anemia and thrombocytopenia in this patient. In the follow-up period, features of myelodysplastic syndrome became

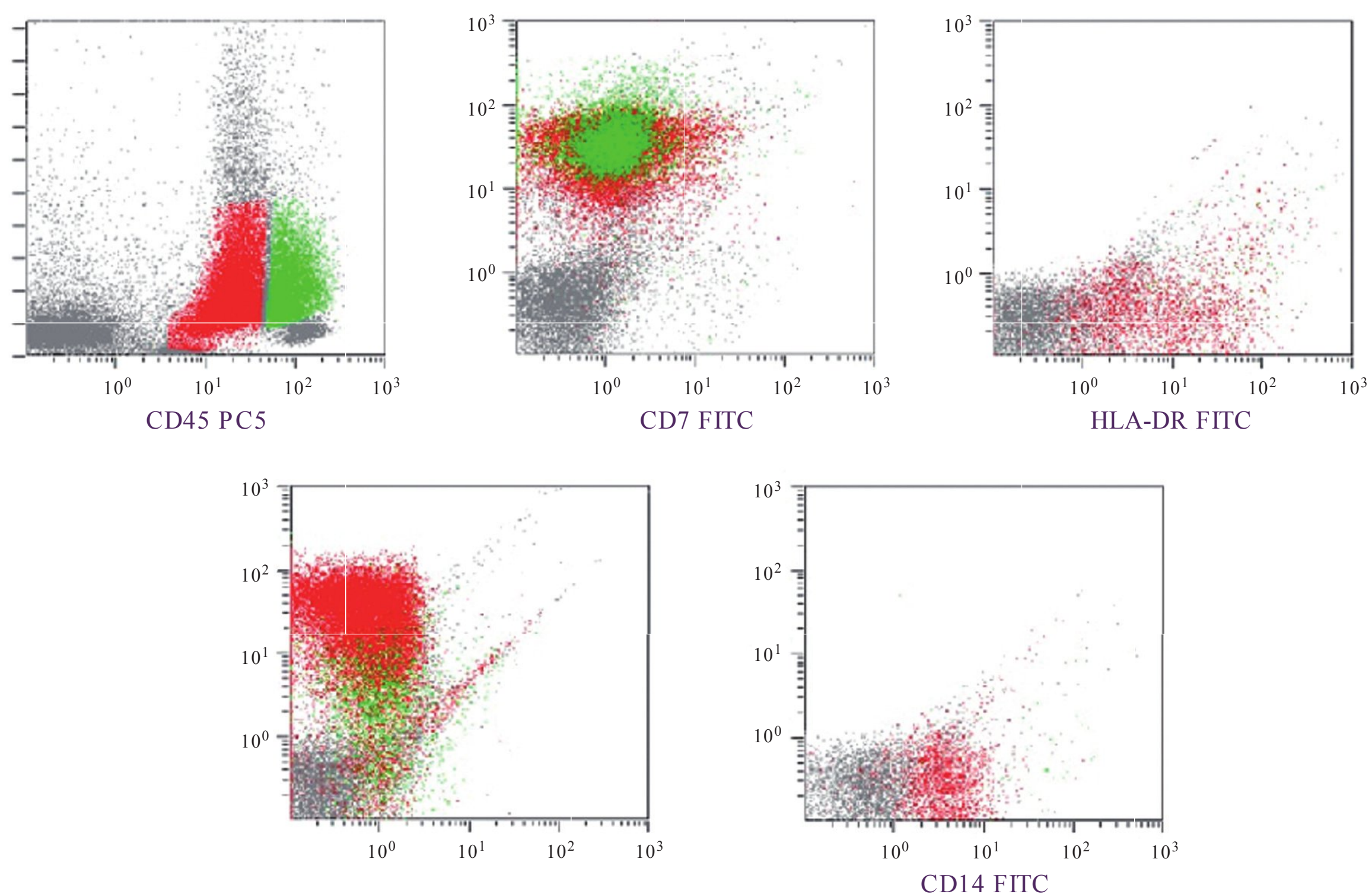


FIGURE 6.10.4 Flow cytometric histograms from a case of acute myelomonocytic leukemia (not the current case) demonstrated two immature cell clusters in the dot plot. Both populations express CD117, CD13-CD33, and (partially) CD7. The red cluster also shows CD14 and HLA-DR. PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; SS, side scatter.

apparent, which rapidly evolved into AML that was composed of both myeloid and monocytoid elements. Based on the bone marrow differential count, the cytochemical findings, and flow cytometric results, this case fulfills the diagnostic criteria of M4.

Morphology and Cytochemistry

The leukemic component in M4 includes type I and type II myeloblasts, monoblasts, and promonocytes (4). Type I myeloblasts have no cytoplasmic granules, and type II myeloblasts have <20 azurophilic granules. Monoblasts and monocytes may or may not have cytoplasmic granules, but their nuclei differ from those of myeloblasts by the folded or lobulated configuration. However, the very immature monoblast may show round or oval nuclei that are similar to those of myeloblasts, but monoblasts are usually larger than myeloblasts and have abundant cytoplasm with irregular border.

Cytochemical stains are originally required by the FAB system for estimation of the percentages of these two populations, because the distinction between myeloblasts and monoblasts is sometimes difficult. However, with the recent development of flow cytometry and immunohistochemistry, cytochemistry is gradually being replaced.

The distinction between monoblasts and promonocytes depends on the nuclear configuration, the chromatin pattern, and the prominence of nucleoli. Promonocytes usually have more obvious lobulation of the nuclei, more mature chromatin pattern, and less conspicuous nucleoli than the monoblasts have.

In the leukemic population, the myeloblasts are positive for MPO, Sudan black B, and CAE. The monoblasts and promonocytes are positive for nonspecific esterases, which include NBE and α -naphthyl acetate esterase. Monocytic series react either weakly positive or negative to MPO and Sudan black B stains.

In some cases of M4, the leukemic cells may show both CAE and NBE in the same cells (7,8) (Fig. 6.10.5).

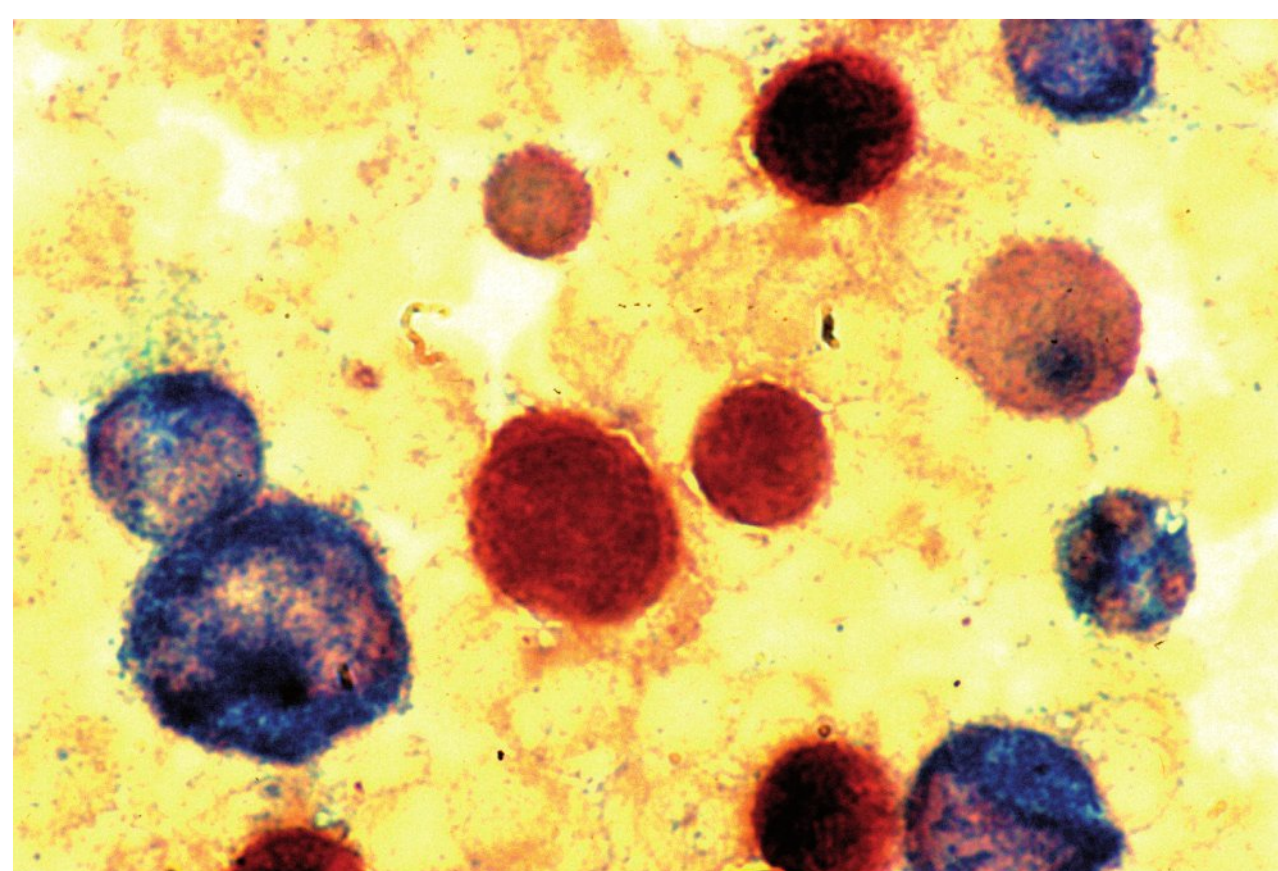


FIGURE 6.10.5 A bone marrow aspirate reveals CAE-positive (blue) and α -NBE-positive (brown) populations. Note that some cells are positive for both esterases. Combined esterase stain, 100 $\times$ magnification. (From Sun T. Flow cytometric analysis of hematologic neoplasms, 2002.)

This population is considered to be a group of hybrid monocyte-granulocyte. Monocytic components in M4 also show double staining of lactoferrin and lysozyme, characteristic of granulocytes (9). Therefore, the myelocytic and monocytic components in M4 are probably derived from the same precursor cells (9).

A subtype of M4 shows bone marrow eosinophilia. This subtype accounts for 15% to 30% of M4 cases (10) and is designated M4 with eosinophilia (M4Eo). M4Eo is associated with a special cytogenetic karyotype: inv(16) or, less frequently, t(16;16). This entity is described in Case 5.

Immunophenotype

The immunophenotypes of various subtypes of AML were delineated by several groups in the late 1980s (11–13). Since then, flow cytometry has become the mainstay for the subclassification. The major myelomonocytic markers are CD13 and CD33, which have been routinely used for screening of myelomonocytic cells. Recently, cytoplasmic staining of MPO also has been included in the panel for AML. The monocytic component, as seen in M4 and M5, is routinely scanned by CD14, which includes several monoclonal antibodies from different manufacturers, such as MO2, MY4, and LeuM3. The combined use of MO2, MY4, and CD64 is able to distinguish various maturation stages (14). CD64 is positive for the entire spectrum of monocytes, MY4 for promonocytes and mature monocytes, and MO2 for mature monocytes only.

Other markers, such as CD11b and CD11c, are not as specific for monocytes as CD14, but they are sometimes more sensitive than the latter. CD11b and CD11c are negative for myeloblasts, but can be demonstrated in the more mature forms of myeloid cells. These monocytic markers, however, cannot be relied upon for quantitation; for instance, to distinguish M4 from M5 (12). Other monocyte markers, such as CD32 and CD36, are seldom used for the diagnosis of AML.

In addition to the identification of myeloid and monocytoid lineage, the immunophenotype should include the immature cell markers, such as CD34 and CD117, to identify the malignant nature of the myelomonocytic population (2,15).

HLA-DR is routinely included in the AML panel, because its absence or decrease in percentage is characteristic of acute promyelocytic leukemia (M3). In the microcytic form of M3, the leukemic cells may show monocytoid nuclei; in those cases, M4 and M5 should be included in the differential diagnosis.

A few studies emphasized the prognostic value of some myeloid markers. For instance, My7 (CD13) and My4 (CD14) are predictors for a low rate of complete remission (16,17). A high CD33/CD13 ratio, in contrast, is a favorable prognostic factor (17). A CD17 antigen, which is seldom included in an AML panel, is a predictor for a shorter survival (13).

T-cell antigens have been detected in 44% of M4-M5 cases (CD2 14%, CD4 12%, CD7 36%) in one study (12). In general, CD7 is the most commonly coexpressed lymphoid antigen in AML cases (3); therefore, it becomes a routine component in the AML panel.

Immunohistochemical staining may demonstrate MPO, lysozyme, CAE (Leder stain), CD15, and CD68. There are two clones of CD68: KP-1 is present in both myeloid and monocytoid cells, whereas PG-M1 is specific for monocytes and/or histiocytes (18). Therefore, the use of PG-M1 is most helpful in identifying the monocytic component in M4 (Fig. 6.10.6). The new monocyte marker, CD163, is specific and sensitive in identifying monocytic population in tissue sections (5,19). The immature cell markers, CD34 and CD117, can be used to identify the malignant nature of the myeloid cells, if it is not morphologically apparent. In general, flow cytometry is the preferred technique to immunohistochemistry in diagnosing this subtype of AML.

Molecular Genetics

In typical M4 cases, the most common cytogenetic abnormality is the translocation of 11q23 with other partner chromosomes, which is seen in about 20% of M4 and M5 cases (1). In a recent study of 1897 AML cases, the incidence of 11q23 abnormality in M4, M5a, and M5b is 4.7%, 33.3%, and 15.9%, respectively (20). Molecular studies have identified a human homolog of the *Drosophila trithorax* gene designated mixed-lineage leukemia (MLL) gene, as it can be demonstrated in both acute myeloid and lymphoid leukemias (10).

More than 30 partners of the MLL gene have been described (21). Among these translocations, t(9;11) is most common. In the Cancer and Leukemia Group B study, AML cases with t(9;11) have longer overall survival than that of other 11q23 rearrangement (22). However, another study found no difference in prognosis between t(9;11) and other forms of 11q23 translocation (20). The incidence of AML with MLL rearrangement is significantly higher in therapy-related AML than in de novo AML (20). In general, AML cases with 11q23 abnormality carry an unfavorable prognosis. The most specific genetic aberration, however, is found in the cases of M4Eo, which show inv(16)(p12q22), t(16;16)(p12;q22), or del(16)(q22). This entity is described in Case 6.

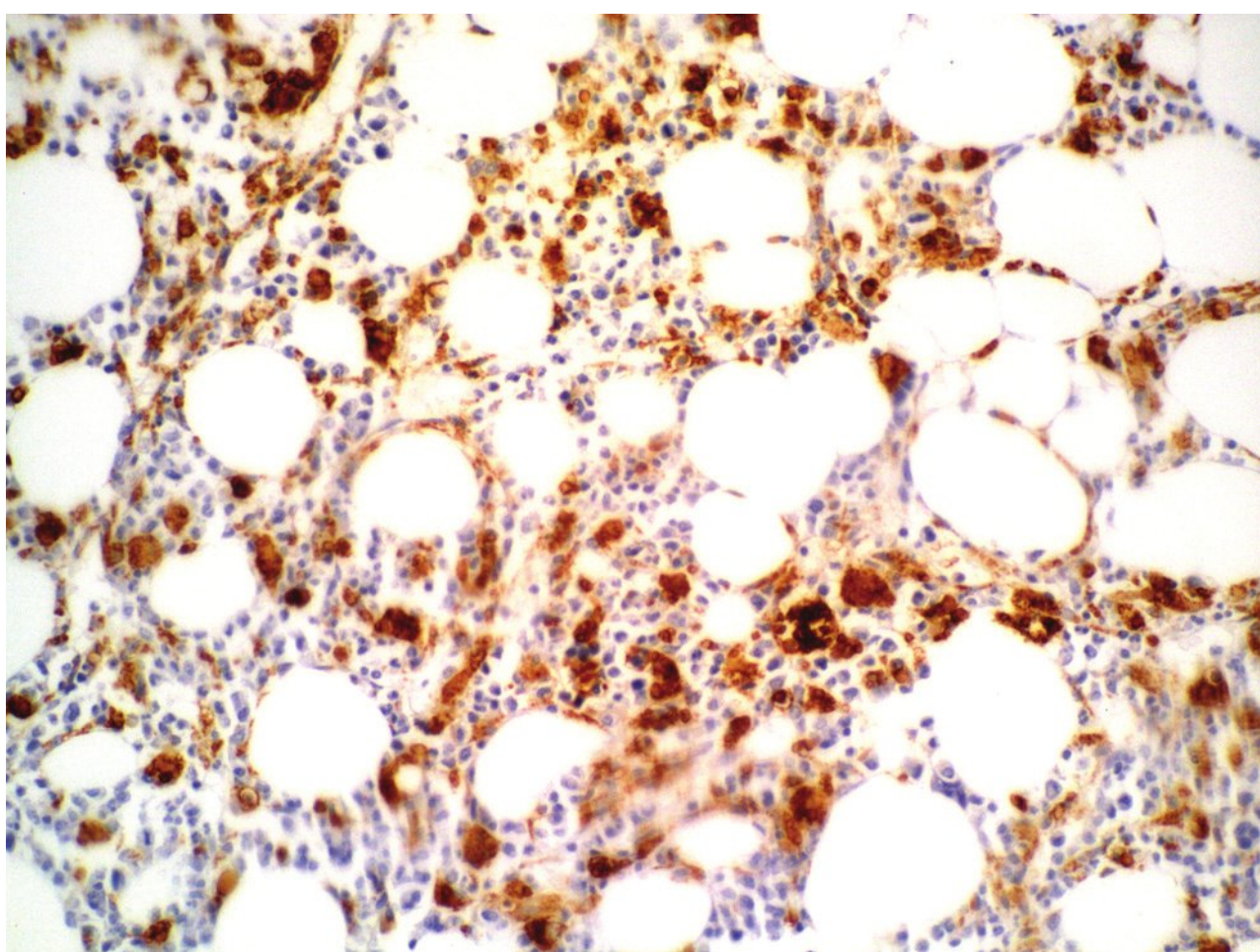


FIGURE 6.10.6 Bone marrow core biopsy shows many cells with CD68 PG-M1 staining. MPO stain, 20× magnification.

An abnormal karyotype, t(6;9)(p21-22;q34) is seen in M4 with basophilia (23). Recently, t(8;16)(p11;p13), or MYST3-CREBBP translocation, has been found in an increasing number of M4 and M5 cases, which are characterized by the presence of erythrophagocytosis in the leukemic blasts (24–26). MYST3-associated AML cases have a specific gene expression profile, which includes overexpression of MYB, CD4, and HOXA genes (27). These features are reminiscent of T-cell acute lymphoblastic leukemia and suggest that these two entities share a common T-myeloid progenitor. Another study of gene expression profiling found that the NF-κB signaling pathway is activated at a higher level in M4Eo than in regular M4 cases (28).

Other low-frequency aberrations include inv(3q26), t(3;3), and +4 (1,23). One study has found high expression of the c-fos protooncogene in M4 and M5 cases (29). N-ras mutation has been reported in a single case of M4 (30).

The salient features for laboratory diagnosis of M4 are summarized in Table 6.10.1.

Clinical Manifestations

M4 occurs in all age groups but is more common in older individuals with the median age of 50 years (2,31). The male/female ratio was 1.4:1 in one study (31).

The clinical presentation is leukocytosis in 85% of patients, and 10% are leukopenic (31). As in the current case, anemia and thrombocytopenia are characteristic features (2). Consequently, patients may have fatigue,

TABLE 6.10.1

Salient Features for Laboratory Diagnosis of AML-M4

1. Presence of at least 20% myeloblasts–monoblasts–promonocytes in bone marrow
2. Both myeloid and monocytoid series should be >20%.
3. If monocytic component is <20% in bone marrow:
 - a. Monocyte count in peripheral blood should be $>5 \times 10^9/L$.
 - b. Serum lysozyme level should exceed three times the normal value.
4. MPO (Sudan black B) positive cells: >3%
5. Specific and nonspecific esterase–positive cells should be >20%.
6. Flow cytometry: positive for CD13, CD14, CD33, HLA-DR, MPO, and one of the stem cell markers (CD34 or CD117)
7. Immunohistochemistry: positive for MPO, lysozyme, CAE, CD15, CD68, CD163, CD34, and CD117
8. Common karyotypes: 11q23 translocation with a partner gene; chromosome 16 abnormalities in M4 with eosinophilia; t(6;9) in M4 with basophilia; t(8;16) in M4/M5 with erythrophagocytosis

AML, acute myeloid leukemia; CAE, chloroacetate esterase; MPO, myeloperoxidase.

fever, bleeding disorders, and gingival hyperplasia. Lymphadenopathy is present in about half the patients and hepatosplenomegaly in 30% to 35% (31). Some patients are preceded with chronic myelomonocytic leukemia (2).

REFERENCES

- Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med*. 1999;341:1051–1062.
- Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukemia not otherwise categorized. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:91–105.
- Smith M, Barnett M, Bassan R, et al. Adult acute myeloid leukaemia. *Crit Rev Oncol Hematol*. 2004;50:197–222.
- Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:626–629.
- Arber DA, Brunning RD, Orazi A, et al. Acute myeloid leukaemia, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al. eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:130–139.
- Moscinski LC, Kasnic G, Saskar A. The significance of an elevated serum lysozyme value in acute myelogenous leukemia with eosinophilia. *Am J Clin Pathol*. 1992;97:195–201.
- Huhn D, Twardzik L. Acute myelomonocytic leukemia and the French-American-British classification. *Acta Haematol*. 1983;69:36–40.
- Li CY, Philyky RL, Yam LT. Acute myelomonocytic leukemia. An unusual variant with both granulocytic and monocytic esterases in the leukemic cells. *Mayo Clin Proc*. 1986;61:104–109.
- Saito N. Acute myelomonocytic leukemia. An immunoelectron microscopic study. *Am J Hematol*. 1990;35:238–246.
- Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia with recurrent genetic abnormalities. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:81–87.
- Neame PB, Soamboonstrup P, Browman GP, et al. Classifying acute leukemia by immunophenotyping. A combined FAB-immunologic classification of AML. *Blood*. 1986;68:1355–1362.
- Schwonzen M, Kuehn N, Vetten B, et al. Phenotyping of acute myelomonocytic (AMMOL) and monocytic leukemia (AMOL). Association of T-cell-related antigens and skin-infiltration in AMOL. *Leuk Res*. 1989;13:893–898.
- Merle-Beral H, Due LNC, Leblond V, et al. Diagnostic and prognostic significance of myelomonocytic cell surface antigens in acute myeloid leukemia. *Br J Haematol*. 1989;73:323–330.
- Yang DT, Greenwood JH, Hartung L, et al. Flow cytometric analysis of different CD14 epitopes can help identify immature monocytic populations. *Am J Clin Pathol*. 2005;124:930–936.
- Hans CR, Finn WG, Singleton TP, et al. Usefulness of anti-CD117 in the flow cytometric analysis of acute leukemia. *Am J Clin Pathol*. 2002;118:31–37.
- Griffin JD, Davis R, Nelson DA, et al. Use of surface marker analysis to predict outcome of adult acute myeloblastic leukemia. *Blood*. 1986;68:1232–1241.
- Kristensen JS, Hokland P. Monoclonal antibodies in myeloid diseases. Prognostic use in acute myeloid leukemia. *Leuk Res*. 1991;15:693–700.
- Knowles DM. Immunophenotypic markers useful in the diagnosis and classification of hematopoietic neoplasms. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:93–226.
- Orazi A, Chiu R, O'Malley DP, et al. Chronic myelomonocytic leukemia: the role of bone marrow biopsy immunohistology. *Mod Pathol*. 2006;19:1536–1545.
- Schoch C, Schnittger S, Klaus M, et al. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. *Blood*. 2003;102:2395–2402.
- Rowley JD. The role of chromosome translocation in leukemogenesis. *Semin Hematol*. 1999;36(suppl 7):59–72.
- Mrozek K, Heinonen K, Lawrence D, et al. Adult patients with de novo acute myeloid leukemia and t(9;11)(p22;q23) have a superior outcome to patients with other translocations involving band 11q 23: a cancer and leukemia group B study. *Blood*. 1997;90:4532–4538.
- Second MIC Cooperative Study Group. Morphologic, immunologic and cytogenetic (MIC) working classification of the acute myeloid leukemias. *Br J Haematol*. 1988;68:487–494.
- Stark B, Resnitzky P, Jeison M, et al. A distinct subtype of M4/M5 acute myeloblastic leukemia (AML) associated with t(8;16)(p11;p13), in a patient with the variant t(8;19)(p11;q13)—case report and review of the literature. *Leuk Res*. 1995;19:367–379.
- Velloso ERP, Mecucci C, Michaux L, et al. Translocation t(8;16)(p11;p13) in acute nonlymphocytic leukemia. Report of two cases and review of the literature. *Leuk Lymphoma*. 1996;21:137–142.
- Sun T, Wu E. Acute monoblastic leukemia with t(8;16). A distinct clinicopathologic entity: report of a case and review of the literature. *Am J Hematol*. 2001;66:207–212.
- Murati A, Gervais C, Carbuca N, et al. Genome profiling of acute myelomonocytic leukemia: alteration of the MYB locus in MYST3-linked cases. *Leukemia*. 2009;23:85–94.
- Sun X, Zhang W, Ramdas L, et al. Comparative analysis of genes regulated in acute myelomonocytic leukemia with and without inv(16)(p13q22) using microarray techniques, real-time PCR, immunohistochemistry, and flow cytometry immunophenotyping. *Mod Pathol*. 2007;20:811–820.
- Mavilio F, Testa W, Sposi NM, et al. Selective expression of fos proto-oncogene in human acute myelomonocytic and monocytic leukemias. A molecular marker of terminal differentiation. *Blood*. 1987;69:160–164.
- Vandenberghe E, Baens M, Stul M, et al. Alteration of N-ras mutation in a patient with AML M4 and trilineage myelodysplasia. *Br J Haematol*. 1991;79:338–340.
- Brunning RD, McKenna RW. *Tumors of the Bone Marrow*. Armed Forces Institute of Pathology (AFIP) Fascicle 9, 3rd series. Washington, DC: AFIP; 1994:51–55.

CASE 11

Acute Monoblastic and Monocytic Leukemia (M5) and 11q23 (Mixed Lineage Leukemia) Abnormalities

CASE HISTORY

A 71-year-old man presented with a history of abdominal pain radiating to his chest for 4 days. The patient had lost 18 lb in the past 6 months. He was diagnosed with adenocarcinoma of the lung by fine-needle aspiration 2 years ago. Because of his seizure activity, the patient had a biopsy of the right parietal lobe, which proved to be metastatic carcinoma of the lung. The patient received local radiation therapy to his thorax and brain at that time. During a visit to the outpatient clinic, the patient was found to have a leukocyte count of 20,500/mL, with 43% segmented neutrophils, 12% bands, 28% lymphocytes, and 15% blasts. He was then scheduled for admission in 2 weeks.

On the day of admission, the leukocyte count rose to 62,700/mL with a differential of 7% segmented neutrophils, 1% bands, 9% lymphocytes, and 81% blasts. Bone marrow examination revealed 95% cellularity, and the aspirate showed 85% monoblasts, 7% promonocytes, and 3% monocytes. Only 5% of normal hematopoietic cells in the myeloid and erythroid cell lines were present.

Because the patient had stage IV lung cancer and type II Mobitz II heart block at the same time, no specific antileukemic treatment was given. He was treated with hydroxyurea to relieve leukostasis and allopurinol to prevent tumor lysis. The patient was discharged and died at home on the day of discharge.

FLOW CYTOMETRY FINDINGS

Bone marrow: Myeloperoxidase (MPO) 30%, CD13-CD33 92%, CD14 48%, CD11c 29%, HLA-DR 87%, CD34 8%, CD7 0% (Fig. 6.11.1).

CYTOCHEMICAL FINDINGS

MPO stain was positive in >10% of blasts. The combined esterase stain showed that >80% blasts in the bone marrow were positive for α -naphthyl butyrate esterase (Fig. 6.11.2).

CYTOGENETIC FINDINGS

Cytogenetic analysis showed two abnormal clones. The first clone of seven cells revealed t(8;16)(p11.2;p13.3), and the second clone of four cells had additional material of

unknown origin on the short arm of the other chromosome 16 in addition to the t(8;16).

DISCUSSION

The French-American-British criteria for the diagnosis of acute monoblastic leukemia (M5) require 80% or more of the nonerythroid cells in the bone marrow to be monoblasts, promonocytes, or monocytes (1). If the predominant component (>80%) is monoblast, the condition is designated M5a, whereas the predominant components should be promonocytes in M5b. The World Health Organization (WHO) Classification adopts the same criteria for the diagnosis of these entities (2). There are no consistent differences in clinical presentation between these two subtypes (3–5). The incidence of these two subtypes combined is approximately 2% to 9% of all cases of acute myeloid leukemia (AML) (6,7).

Morphology

The monoblasts are usually larger than the myeloblasts, measuring about 40 to 50 nm in diameter (Figs. 6.11.3 and 6.11.4). The cytoplasm in most monoblasts is abundant with a grayish-blue tinge. It contains fine or inconspicuous granules, and the cell border is irregular. The nuclei of the very immature monoblasts are round or oval, but folding or creasing is frequently visible in most monoblasts. The promonocyte differs from the monoblast in its smaller size (up to 35 nm) and more prominent folding or creasing or lobulation of the nuclei. The presence of nucleoli in most promonocytes helps distinguish them from mature monocytes. Cytoplasmic vacuolation is frequently seen in monocytic elements. Recently, the International Group on Morphology of Myelodysplastic Syndrome has recommended the addition of a 4th monocytic cell type, the immature monocyte (8). The immature monocytes appear as an abnormal monocyte, but it is distinguished from promonocyte by the presence of more condensed chromatin pattern, rare nucleolus, and less basophilic cytoplasm (8). This distinction is very important for the differential diagnosis between M5 and chronic myelomonocytic leukemia; promonocytes are counted as leukemic blasts but immature monocytes are not.

In general, if leukemic bone marrow shows a great variation in the nuclear configuration from cell to cell, the possible diagnosis of M5 should be considered (Figs. 6.11.4 and 6.11.5). Despite all these characteristics, cytochemical stain should

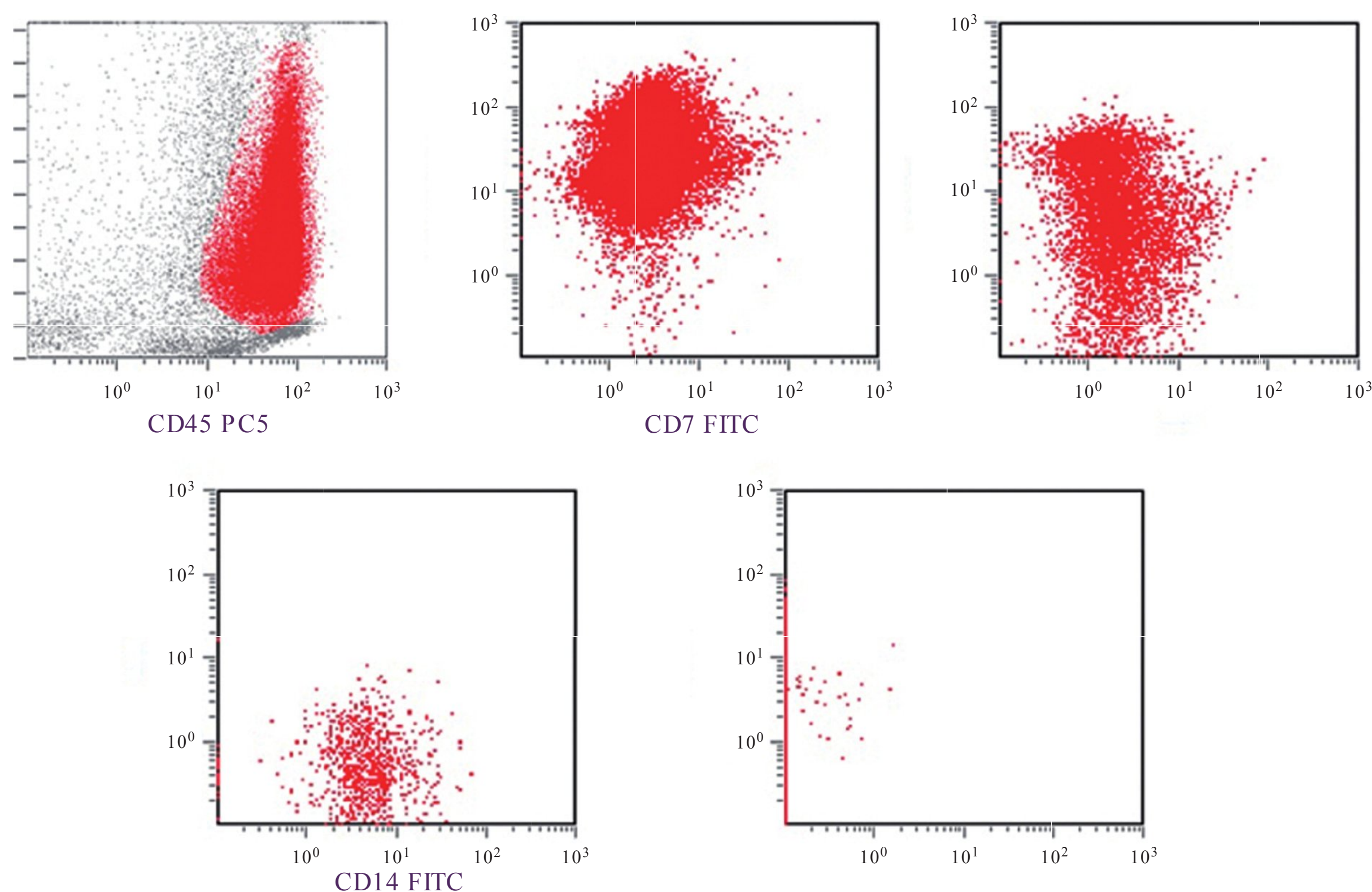


FIGURE 6.11.1 Flow cytometric analysis of bone marrow in an M5 case shows positive reactions to CD7, CD13-CD33, CD14, and CD117, but negative reactions to CD34. These histograms are not from the current case. SS, side scatter; PE, phycoerythrin; PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; RD1, rhodamine.

be done routinely to definitively identify monocytic elements. For instance, neuroblastoma cells may occasionally be mistaken as monoblasts (9).

For cytochemical staining, M5 is an exceptional subtype of AML that is not required to have more than 3% MPO-positive blasts, because MPO is frequently negative in M5

(10,11). However, the MPO-negative cases should have a strong nonspecific esterase staining to back up the diagnosis. Chloroacetate esterase and periodic acid-Schiff are usually negative in M5, but they can be weakly positive in some cases (10,11). One study found that only one half of the M5 cases were positive for both a-naphthyl acetate esterase

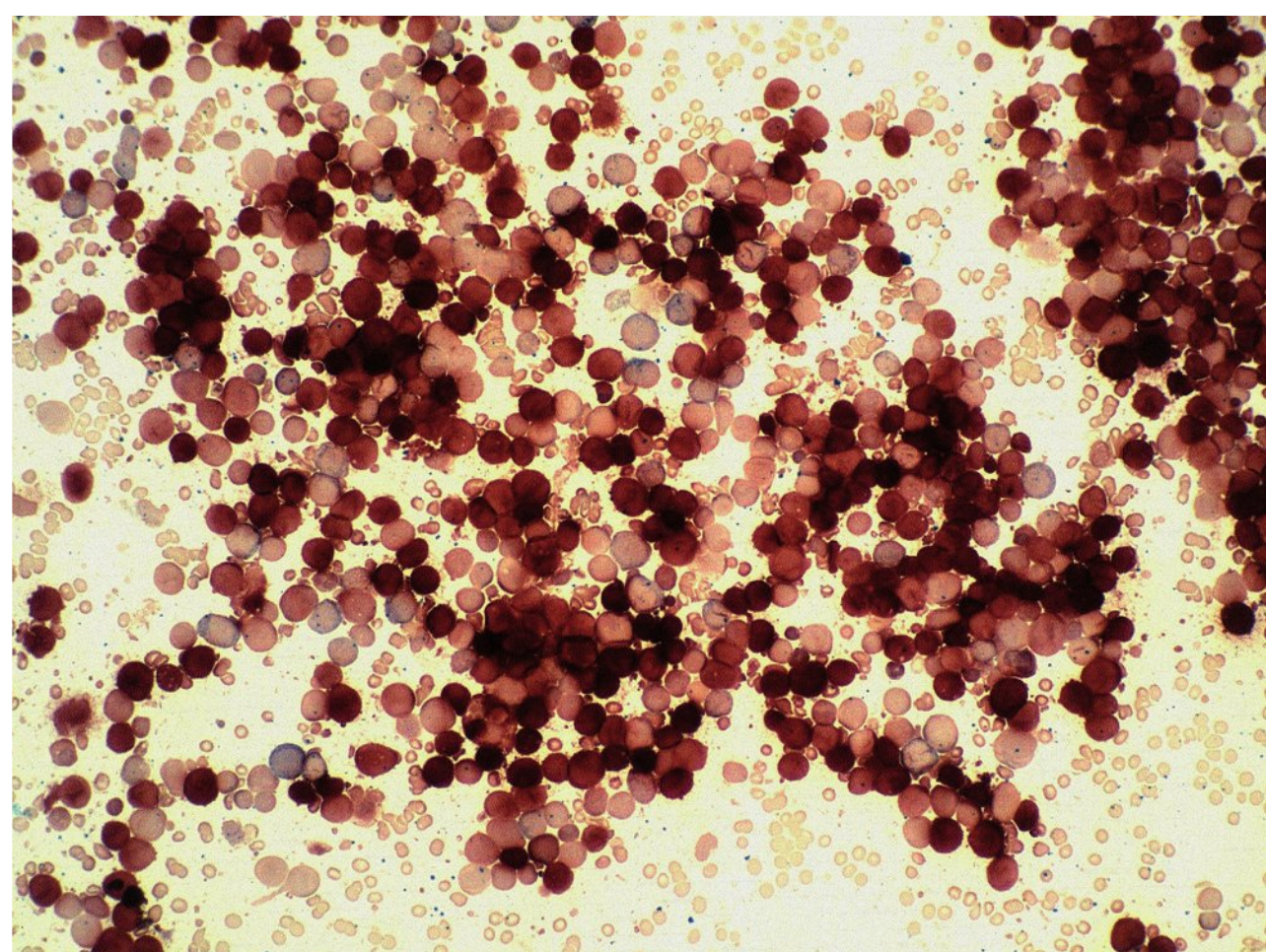


FIGURE 6.11.2 Combined esterase stain of a bone marrow aspirate shows predominantly nonspecific esterase staining of the blasts. Specific esterase stains only a few mature myeloid cells. 40× magnification.

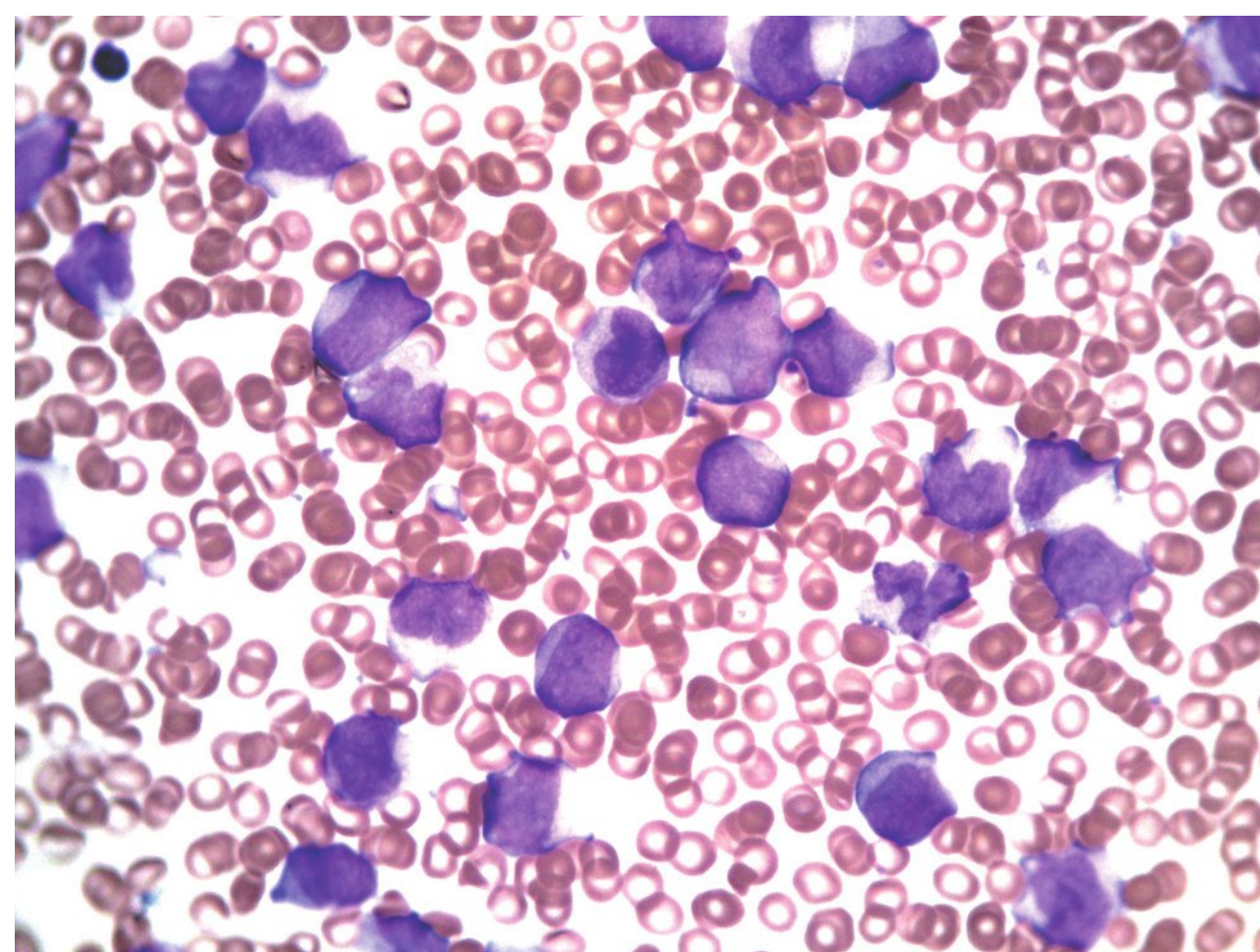


FIGURE 6.11.3 Peripheral blood smear of an M5 case shows various monocytic stages. Wright-Giemsa, 60× magnification.

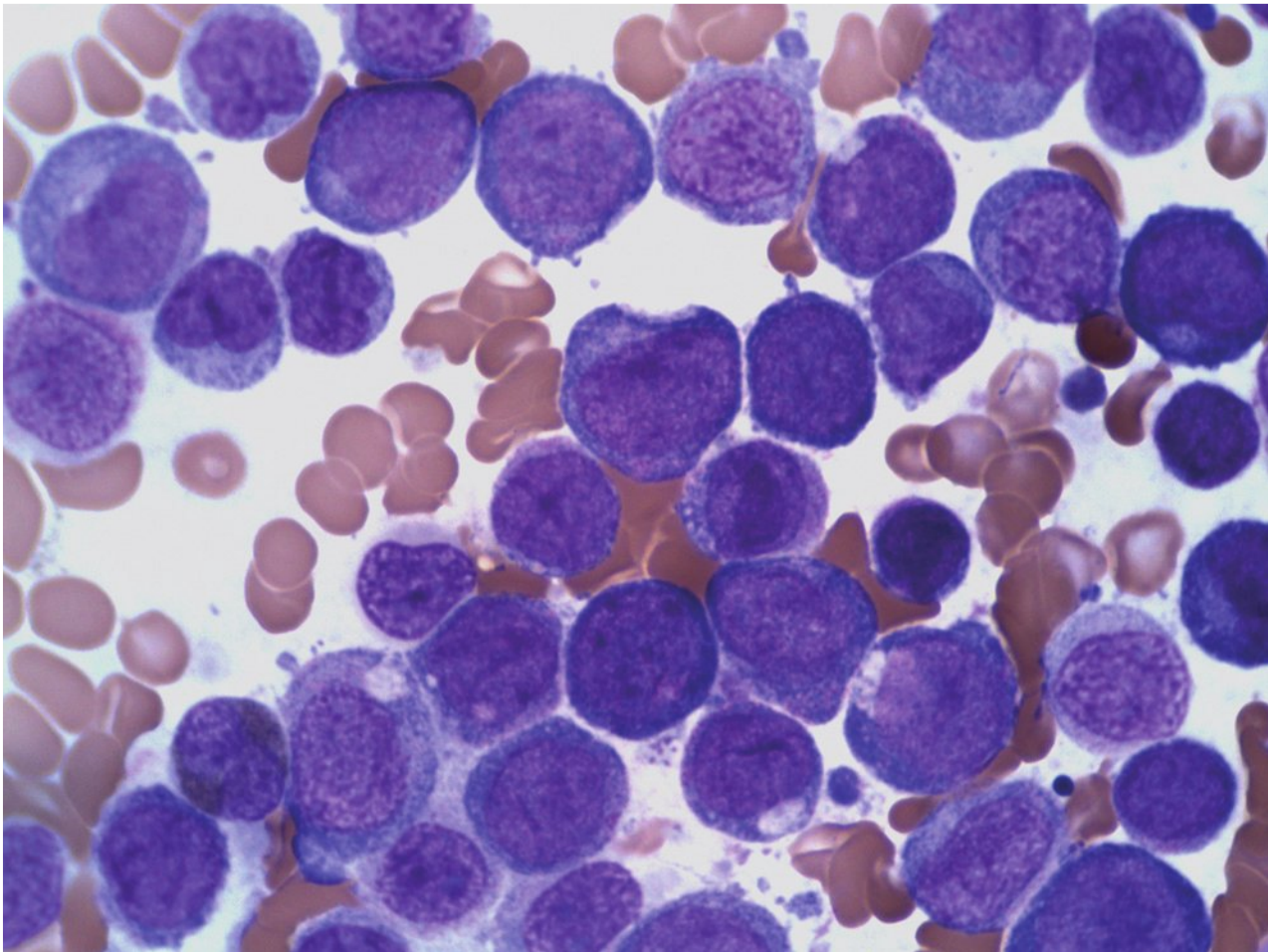


FIGURE 6.11.4 Bone marrow aspirate of an M5 case shows many monoblasts and promonocytes with a few monocytes. Wright-Giemsa, 100× magnification.

and CD14, whereas 25% were positive for α-naphthyl acetate esterase only and another 25% were positive for CD14 only (12). Therefore, a combination of cytochemistry and immunophenotyping is necessary for an accurate diagnosis of M5.

In the current case, the patient's bone marrow contained 95% monocytic cells with 85% monoblasts, which were verified by the nonspecific esterase stain. Therefore, a diagnosis of acute monoblastic leukemia was established. In addition, erythrophagocytosis was demonstrated in the monoblasts (Fig. 6.11.6), and the blasts in the peripheral blood showed prominent cytoplasmic granules (Fig. 6.11.7). These cytologic features are characteristic of a subtype of AML (usually M4 or M5) with the t(8;16) translocation, as documented in our case. As will be discussed later, this karyotype is not very common, but it is worth identifying

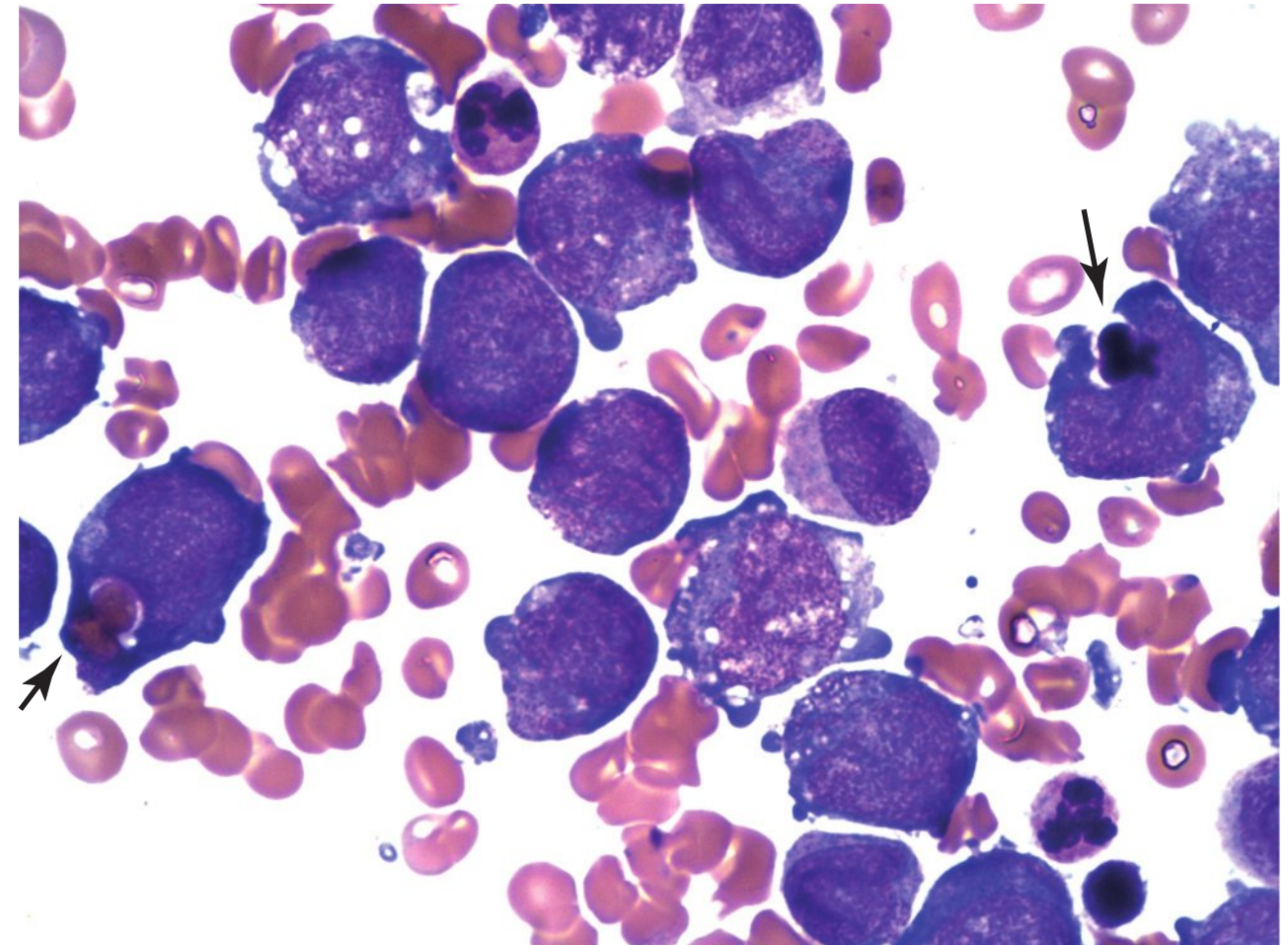


FIGURE 6.11.6 Bone marrow aspirate of an M5 case with t(8;16) shows phagocytosis of erythrocytes and normoblasts by monoblasts (arrows). Wright-Giemsa, 100× magnification.

because it carries a particularly poor prognosis, especially in treatment-related or secondary leukemia (13,14).

Immunophenotype

The immunophenotype of M5 is composed of two sets of antigens. The first set is myeloid markers, which include CD13, CD15, CD33, and HLA-DR (15). However, myeloid markers, such as CD13 and CD15, can be selectively lost in M5 cases (16). The second set is monocyte markers that include CD4, CD11b, CD11c, CD14 (My4, LeuM3, and Mo2), CD32, CD36, CD64, CD68, CD163, and lysozyme. However, CD32 and CD36 are seldom used in routine testing (17,18).

CD34, the stem cell marker, is frequently negative in M5 (15,19), so CD117 is very important to identify the immature cells and to establish the diagnosis of leukemia. Previous studies have emphasized the percentage of CD14

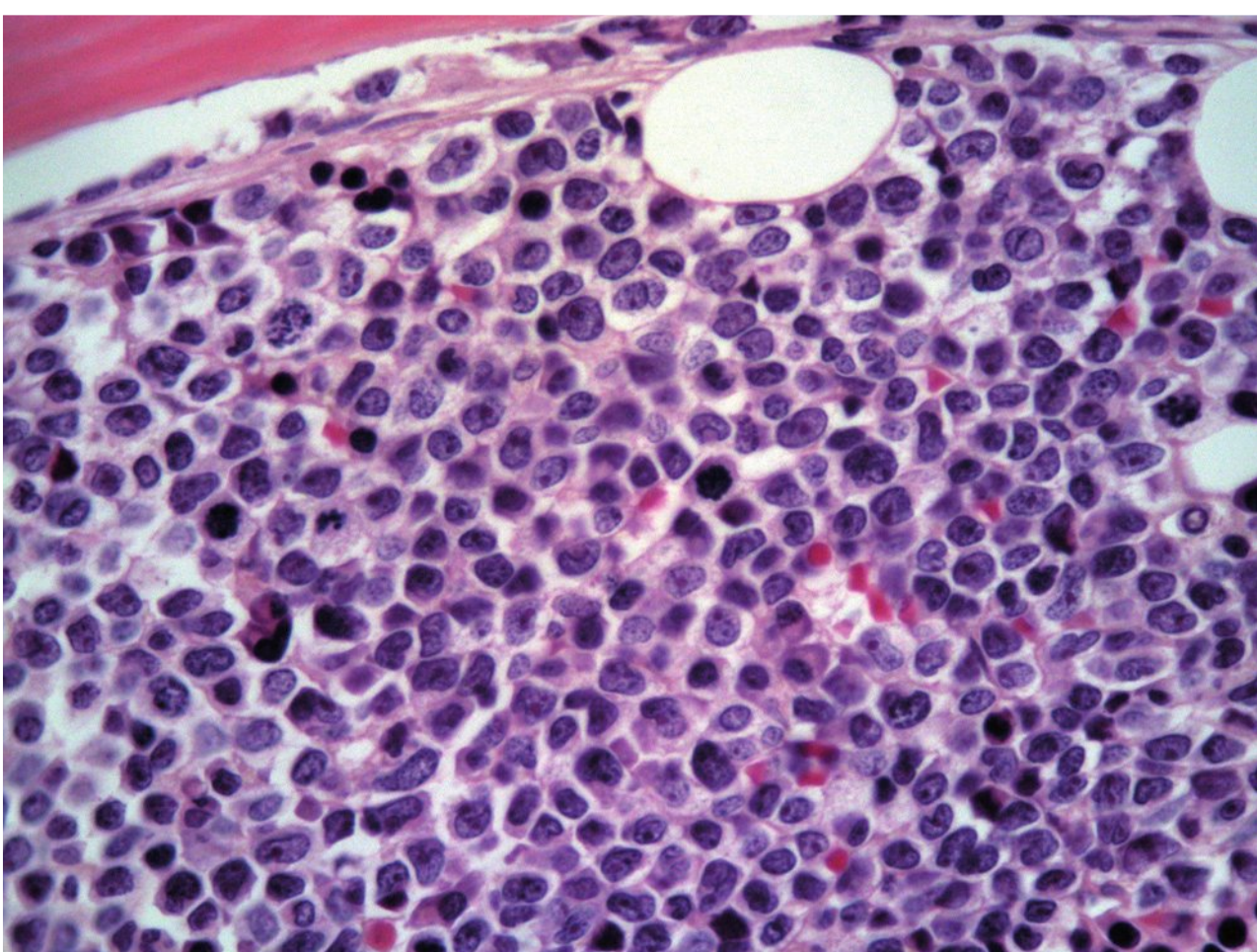


FIGURE 6.11.5 Bone marrow biopsy of an M5 case shows various stages of monocytes, replacing the normal hematopoietic cells. Hematoxylin and eosin, 60× magnification.

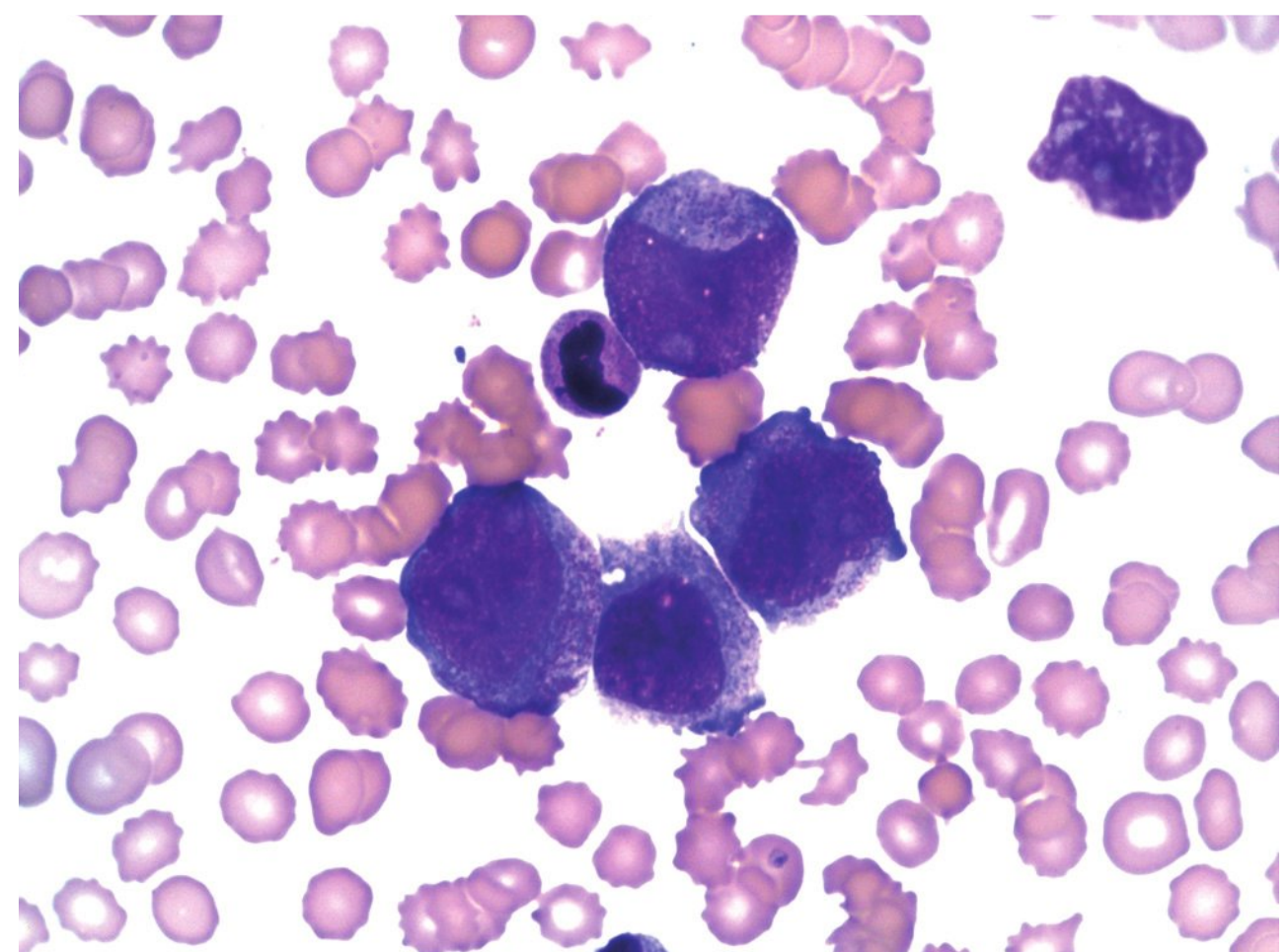


FIGURE 6.11.7 Peripheral blood smear of an M5 case with t(8;16) shows hypergranular monoblasts. Wright-Giemsa, 100× magnification.

expression as the major tool for distinction between M4 and M5 (20), but this assertion was subsequently challenged by others (21,22).

Although M5a and M5b share all antigens, some antigens are preferentially expressed in mature cells whereas others are demonstrated in immature cells. For instance, CD4 and CD14 are mainly demonstrated in mature monocytes, so they are often present in M5b cases (4,23). In contrast, CD117 is shown mainly in immature monocytes (23). CD11b and CD11c are present in both mature and immature monocytes; therefore, an immunophenotype of CD14⁺, CD11b⁺, and CD117⁺ is often seen in M5a cases (5). A recent study demonstrates that the combined use of different CD14 epitopes (Mo2 and My4) and CD64 can stratify different maturation stages of monocytes; this strategy appears to be useful in separating M5a and M5b (24). CD64 is positive for all mature and immature monocytes. My4 is present in mature monocytes and promonocytes, whereas Mo2 is only expressed by mature monocytes (24).

Immunoperoxidase antigen can be demonstrated by flow cytometry in M5b cases, but less often in M5a cases (15). However, immunoperoxidase activity is seldom demonstrated by cytochemistry in M5 cases.

CD56, a natural killer (NK) cell marker, is frequently positive in M5 cases, even though it is not specific (16,25). In one study, CD56 was demonstrated in 86% of M5 cases, just second to blastic NK cell lymphoma and/or leukemia in frequency and far higher than any other AMLs (19). Another study showed CD56 in all 27 M5 cases studied (26).

Lymphoid markers can be detected in certain percentages of M5 cases. T-cell markers (CD2 and CD7) have been demonstrated in M5 cases (19,21). CD20 and CD23 have also been detected in a subpopulation of blast cells in M5 (19,23). Cases with a CD14-negative and T-cell antigen-positive phenotype are associated with leukemic skin infiltration (21). Platelet-megakaryocyte antibodies (CD41, CD42, and CD61) and antierythroid antibody (antiglycophorin A) do not cross-react with M5 cells. However, CD36, another platelet antigen, has been consistently demonstrated in both M5a and M5b cases (23).

A relatively new marker for monocytic lineage is CD163, which is the hemoglobin scavenger receptor. It can be demonstrated in M4 and M5 cases, but is seldom present in other AMLs (27).

Comparison of Flow Cytometry and Immunohistochemistry

For the diagnosis of M5, the most difficult part is the identification of the monocytic series. Flow cytometry is able to demonstrate several markers, including CD4, CD11b, CD11c, CD14, and CD64. CD68 is most frequently used in immunohistochemistry, but two clones of CD68 are available. The KP-1 antibody is positive for both myeloid and monocytic cells, whereas PG-M1 is specific for monocytes (Fig. 6.11.8). Lysozyme stain can also be used in tissue stain, but it can be present in both monocytes and myelocytes. The new marker, CD163, is very sensitive in identifying monocytic population in the bone marrow by immunohistochemical staining. If the cell lineage cannot be clearly identified by immunophenotyping, cytochemistry is the final resort.

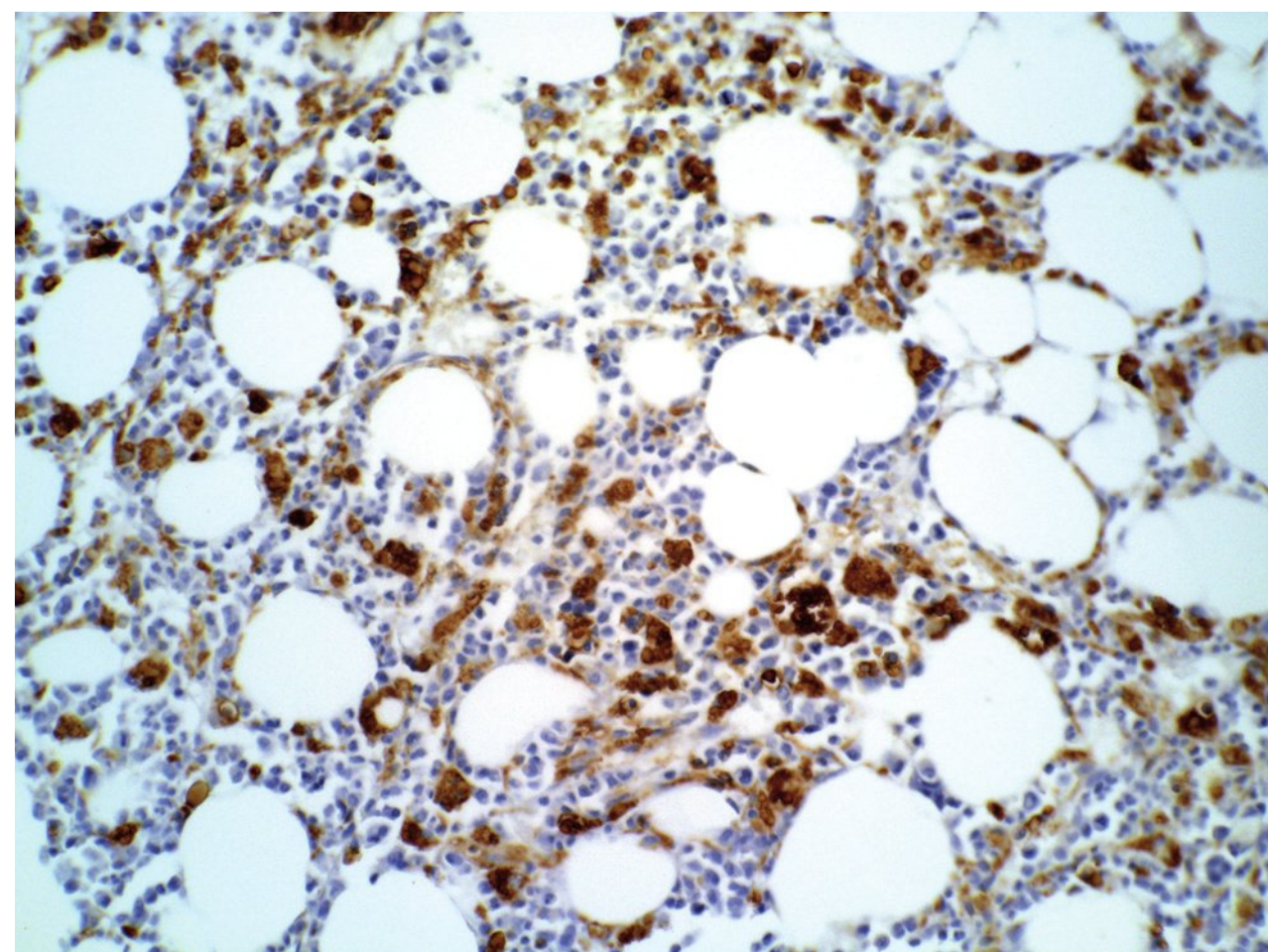


FIGURE 6.11.8 Bone marrow biopsy of an M5 case shows extensive staining of CD68 PG-M1. Immunoperoxidase, 20× magnification.

Both a-naphthyl butyrate esterase and a-naphthyl acetate esterase are specific for monocytic series, if the stains are inhibited by sodium fluoride.

Molecular Genetics

The most common cytogenetic aberration in M5 involves 11q23; it accounts for 20% of abnormalities in M5 (28). In the WHO classification, 11q23 abnormalities are grouped together as a separate entity, but these abnormalities are mainly present in M5 cases.

Although >30 partner genes have been found participating in translocation of 11q23, several chromosomal loci, including 6q27, 9q22, 10p12, 27q21, and 19p13.1, are frequently involved (5,28–31). The t(6;11) is usually seen in young men who present clinically with localized infection and a moderate leukocytosis (30).

The gene located at 11q23 was originally called ALL1, but it was later found that ALL1 is the same as MLL (mixed lineage leukemia) gene (31). Because 11q23-involved abnormalities have been found in AML, acute lymphoblastic leukemia, and lymphomas, MLL is the preferred term to use.

In a study of 58 M5a and 66 M5b patients, 11q23/MLL aberrations were detected in 31% of M5a cases and 12.1% of M5b cases (32). The second most common cytogenetic abnormality in this study was sole trisomy 8, which was found in 22.4% of M5a and 3% of M5b cases (32) and 0.2% to 0.4% among all AML cases (33).

Another cytogenetic abnormality, t(8;16)(p11;p13), is only seen in 2% of M5 cases (25), but it is associated with highly specific pathologic findings, namely, erythrophagocytosis by leukemic blasts and hypergranulation in monocytic components (11,34–36). The gene located at 8p11 is MOZ (monocytic leukemia zinc finger), and that at 16p13 is CBP (cAMP response element binding [CREB]-binding protein). Normally, MOZ protein interacts with its cognate complex and joins the general transcription apparatus complex to direct transcription of a certain gene (37). CBP protein, in contrast, interacts with an appropriate



DNA-binding factor and the transcriptional apparatus complex to direct transcription of another gene (37). As a result of the translocation, the fusion product of these two genes (MOZ/CBP) may lead to leukemogenesis through three possible mechanisms: the fusion product mistargets the wrong gene instead of the genes these two individual proteins (MOZ and CBP) are supposed to target, the fusion product misregulates the targeted gene(s), and the fusion product loses normal function in directing DNA transcription (37). There is an interesting observation that cases with *inv*(8)(p12q13), which fuses the MOZ with NCOA2 (nuclear receptor coactivator 2), also show erythrophagocytosis by the leukemic cells (33). It implies that erythrophagocytosis is associated with MOZ involvement. Gene expression profiling showed that AML with *t*(8;16) cases were clustered close to *t*(11q23)/MLL, but were distinct from *t*(15;17), *t*(8;21), and *inv*(16) cases (33).

In patients with a normal karyotype, partial tandem duplication of the MLL gene (MLL-PTD) was identified in 1.7% of M5a cases and 4.5% of M5b cases by reverse transcriptase–polymerase chain reaction (RT-PCR) and was confirmed by Southern blot (32). The frequency of Fms-like tyrosine kinase 3 length mutations (FLT3-LM) was detected by the same techniques in 6.9% of M5a and 28.8% of M5b cases (32). The FLT3 gene mutations in other studies were reported to be as high as 40% (5).

The salient features for laboratory diagnosis of M5 are summarized in Table 6.11.1.

Clinical Manifestations

M5 is predominantly seen in adults older than 40 years and in children younger than 10 years (50% of M5 patients are younger than 2 years) (38). Congenital M5 cases have been reported from time to time (38–41). The occurrence of M5 in young children appears to be associated with in utero exposure to pesticides and solvents (38). Pediatric patients usually have a worse prognosis than adult patients have.

M5 cases generally have higher leukocyte and platelet counts and more frequent lymphadenopathy than do other subtypes of AML (3,38). Disseminated intravascular coagulation is frequently seen in M5, second only to M3 (3,38). The lysozyme level is elevated in 67% to 100% of M5 cases. As lysozyme is nephrotoxic, 40% of M5 patients in one study had renal insufficiency, which was proportional to the lysozyme levels (42). About one fourth of patients with M5 have leukemic infiltration of skin (Fig. 6.11.9) and/or gum, one half have hepatosplenomegaly with or without lymphadenopathy, 3% to 22% have central nervous system involvement, and 28% have renal failure (3,4,43).

As a result of all these complications, M5 patients have a shorter survival time than patients with other subtypes of AML, although the complete remission rate may be comparable (3,44). The three major factors affecting the survival time in M5 cases are age, renal failure, and serum b_2 microglobulin (B_2M) levels, as demonstrated in one study (4). Patients with renal failure or high B_2M levels have a median survival of 1 and 3 weeks, respectively, whereas patients with no renal failure or low B_2M levels have a median survival of 26 and 29 weeks, respectively. In the same study, lysozyme, lactate dehydrogenase, and B_2M levels were

TABLE 6.11.1

Salient Features for Laboratory Diagnosis of M5

- 1. Presence of >80% monocytic components among the nonerythroid cells in the bone marrow
 - a. M5a: $\geq 80\%$ of monocytic components are monoblasts.
 - b. M5b: predominantly monocytes and promonocytes
- 2. Elevation of serum and urine lysozyme levels
- 3. Cytochemistry
 - MPO: may or may not be positive
 - Nonspecific esterase: strongly positive
 - Specific esterase and periodic acid-Schiff: usually negative
- 4. Immunophenotype
 - Myeloid markers: CD33, CD13, CD15 may be positive, but one or more markers may be selectively lost.
 - Monocytic markers: CD4, CD11b, CD11c, CD14, CD64, CD68, CD163, and lysozyme may be positive, but one or more markers may be selectively lost.
 - Immature cell markers: CD34 is usually negative, and CD117 is frequently positive.
 - Frequently positive nonspecific marker: CD56
 - Consistently negative markers: CD41, CD42, CD61, glycophorin A
- 5. Cytogenetics: associated with *t*/del(11)(q23), *t*(8;16) (p11;p13), or others
- 6. Molecular biology: MLL gene on 11q23 and MOZ/CBP on 8p11/16p13
 - FLT3 gene mutation is a frequent finding in patients with a normal karyotype.

MLL, mixed lineage leukemia; MOZ/CBP, monocytic leukemia zinc finger/CREB-binding protein; FLT3, Fms-like tyrosine kinase 3.

elevated in 88%, 68%, and 81% of M5 cases, respectively, but the first two components did not show statistically significant association with patient prognosis (4).

A recent study considered the poor prognosis of M5 associated with cytogenetic abnormalities, such as mutations in the FLT3 genes (5). This study also found that the disease-free survival and overall survival of patients with M5 in general did not appear to differ from non-M5 AML cases with currently available therapy.

The clinical symptoms in patients with *t*(8;16) are similar to those without this aberration. However, patients with *t*(8;16) have a higher frequency of coagulopathy and extramedullary dissemination (13,34–36). The cytochemistry of leukemic cells is characterized by the positive staining with both MPO and nonspecific esterase (33). In addition, cases with *t*(8;16) are more frequently seen in therapy-related

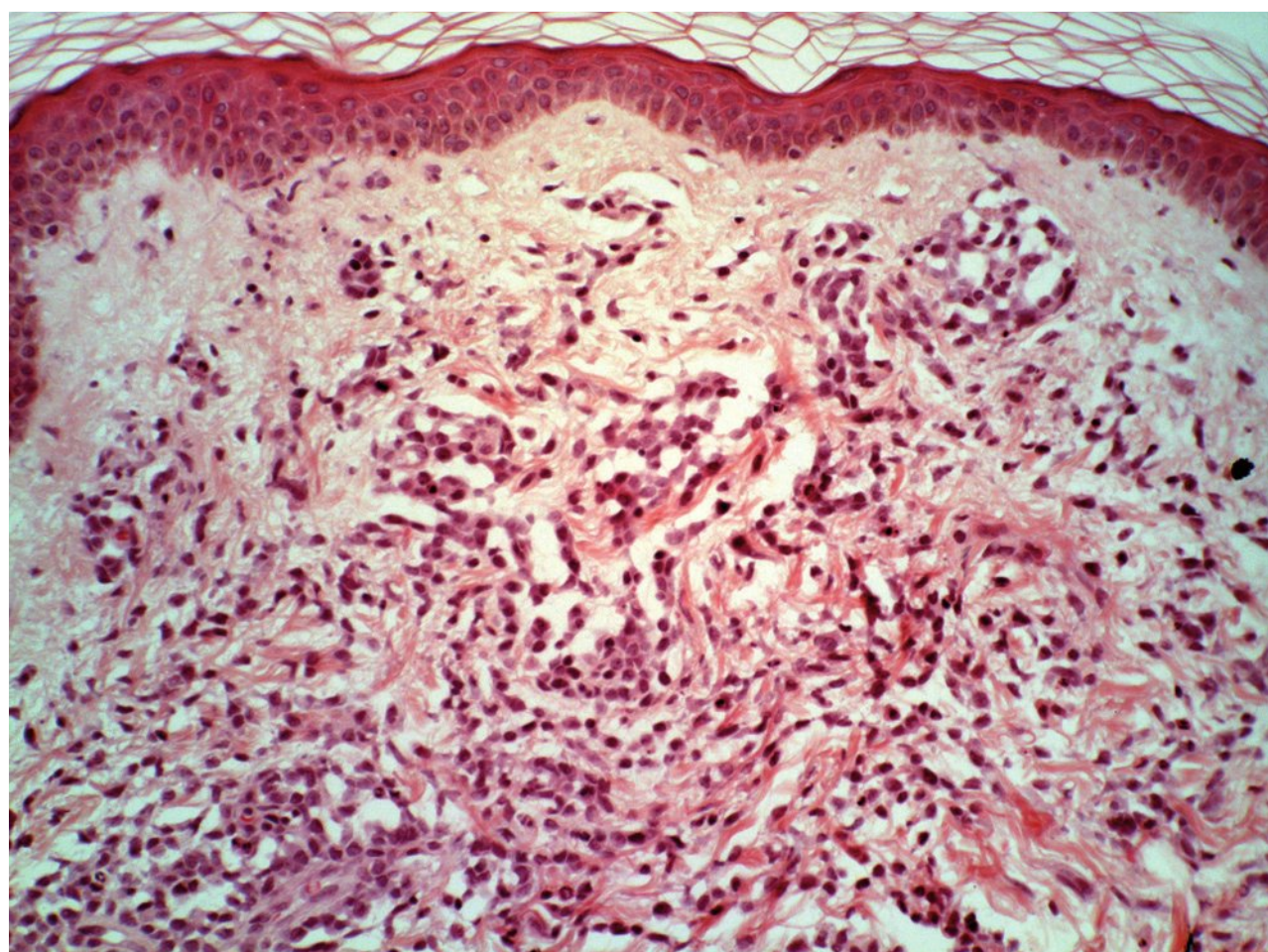


FIGURE 6.11.9 Skin biopsy of an M5 case shows extensive leukemic cell infiltration in the dermis. Hematoxylin and eosin, 20× magnification.

AML than in de novo AML, and this correlation probably explains the poor prognosis in this subtype of AML with a median overall survival of 4.7 months in a report of 13 cases (33).

REFERENCES

- Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia. A report of the French-American-British Cooperative Group. *Ann Intern Med.* 1985;103:620–625.
- Arber DA, Brunning RD, Le Beau MM, et al. Acute monoblastic and monocytic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al. eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed., Lyon, France: IARC Press; 2008:133–134.
- Peterson BA, Levine EG. Uncommon subtypes of acute non-lymphocytic leukemia: clinical features and management of FAB M5, M6, and M7. *Semin Oncol.* 1987;14:425–434.
- Scott CS, Stark AN, Limbert HJ, et al. Diagnostic and prognostic features in acute monocytic leukemia: an analysis of 51 cases. *Br J Haematol.* 1988;69:247–252.
- Tallman MS, Kim HT, Paietta E, et al. Acute monocytic leukemia (French-American-British classification M5) does not have a worse prognosis than other subtypes of acute myeloid leukemia: a report from the Eastern Cooperative Oncology Group. *J Clin Oncol.* 2004;22:1276–1286.
- Flandrin G, Bernard J. Cytological classification of acute leukemias. A survey of 1400 cases. *Blood Cells.* 1975;1:7–15.
- Petti MC, Anadori S, Annino L, et al. Clinical and biological aspects of acute monocytic leukemia (a retrospective study of 29 patients). *Haematologica.* 1982;67:556–566.
- Goasguen JE, Bennett JM, Bain BJ, et al. Morphological evaluation of monocytes and their precursors. *Haematologica.* 2009;94:994–997.
- Boyd JE, Parmley RT, Langevin AM, et al. Neuroblastoma presenting as acute monoblastic leukemia. *J Pediatr Hematol Oncol.* 1966;18:206–212.
- Goasguen JE, Bennett JM. Classification of acute myeloid leukemia. *Clin Lab Med.* 1990;10:661–681.
- Elghetany MT, MacCallum JM, Davey FR. The use of cytochemical procedures in the diagnosis and management of acute and chronic myeloid leukemia. *Clin Lab Med.* 1990;10:707–720.
- Milligan DW, Roberts BE, Limbert HJ, et al. Cytochemical and immunological characteristics of acute monocytic leukemia. *Br J Haematol.* 1984;58:391–397.
- Sun T, Wu E. Acute monoblastic leukemia with t(8;16): a distinct clinicopathologic entity; report of a case and review of the literature. *Am J Hematol.* 2001;207–212.
- Tasaka T, Matsushashi Y, Uehara E, et al. Secondary acute monocytic leukemia with a translocation t(8;16)(p11;p13): case report and review of the literature. *Leuk Lymphoma.* 2004;45:621–625.
- Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia not otherwise categorized. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:91–105.
- Dunphy CH, Orton SO, Mantell J. Relative contributions of enzyme cytochemistry and flow cytometric immunophenotyping to the evaluation of acute myeloid leukemias with a monocytic component and of flow cytometric immunophenotyping to the evaluation of absolute monocytosis. *Am J Clin Pathol.* 2004;122:865–874.
- Merle-Beral H, Due LNC, Leblond V, et al. Diagnostic and prognostic significance of myelomonocytic cell surface antigens in acute myeloid leukemia. *Br J Haematol.* 1989;73:323–330.
- Ball ED. Immunophenotyping of acute myeloid leukemia cells. *Clin Lab Med.* 1990;10:721–736.
- Gorczyca W. Flow cytometry immunophenotypic characteristics of monocytic population in acute monocytic leukemia (AML-M5), acute myelomonocytic leukemia (AML-M4), and chronic myelomonocytic leukemia (CMML). *Methods Cell Biol.* 2004;75:665–677.
- Neame PB, Soamboonsrup P, Browman GP, et al. Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML. *Blood.* 1986;68:1355–1362.
- Schwonzen M, Juehn N, Vetten B, et al. Phenotyping of acute myelomonocytic (AMMOL) and monocytic leukemia (AMOL): association of T-cell related antigens and skin infiltration in AMOL. *Leuk Res.* 1989;13:893–898.
- Drexler HG. Classification of acute myeloid leukemias—FAB or immunophenotyping. *Leukemia.* 1987;1:697–705.
- Khalidi H, Medeiros LJ, Chang K, et al. The immunophenotype of acute myeloid leukemia: high frequency of lymphoid antigen expression and comparison of immunophenotype, French-American-British classification, and karyotypic abnormalities. *Am J Clin Pathol.* 1998;109:211–220.
- Yang DT, Greenwood JH, Hartung L, et al. Flow cytometric analysis of different CD14 epitopes can help identify immature monocytic populations. *Am J Clin Pathol.* 2005;124:930–936.
- Tauchi T, Ohyashiki K, Ohyashiki JH, et al. CD4+ and CD56+ acute myeloblastic leukemia. *Am J Hematol.* 1990;34:228–229.
- Kern W, Bacher U, Haferlach C, et al. Acute monoblastic/monocytic leukemia share common immunophenotypic features but differ in the extent of aberrantly expressed antigens and amount of granulocytic cells. *Leuk Lymphoma.* 2011;52:92–100.
- Walter RB, Bachli EB, Schaer DJ, et al. Expression of the hemoglobin scavenger receptor (CD163/HbSR) as

- immunophenotypic marker of monocytic lineage in acute myeloid leukemia. *Blood*. 2003;101:3755–3756.
28. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. *N Engl J Med*. 1999;341:1051–1062.
 29. Berger R, Le Coniat M, Flexor MA, et al. Translocation t(10;11) involving the MLL gene in acute myeloid leukemia. Importance of fluorescence in situ hybridization (FISH) analysis. *Ann Genet*. 1996;39:147–151.
 30. Welborn JL, Jenks HM, Hagemeijer A. Unique clinical features and prognostic significance of the translocation (6;11) in acute leukemia. *Cancer Genet Cytogenet*. 1993;65:125–129.
 31. Caligiuri MA, Strout MP, Gilliland DG. Molecular biology of acute myeloid leukemia. *Semin Oncol*. 1997;124:32–44.
 32. Haferlach T, Schoch C, Schnittger S, et al. Distinct genetic patterns can be identified in acute monoblastic and acute monocytic leukemia (FAB AML M5a and M5b): a study of 124 patients. *Br J Haematol*. 2002;118:426–413.
 33. Haferlach T, Kohlmann A, Klein HU, et al. AML with translocation t(8;16)(p11;p13) demonstrates unique cytomorphological, cytogenetic, molecular and prognostic features. *Leukemia*. 2009;23:934–943.
 34. Hanslip JJ, Swansbury GJ, Pinkerton R, et al. The translocation t(8;16)(p11;p13) defines an AML subtype with distinct cytology and clinical features. *Leuk Lymphoma*. 1992;6:479–486.
 35. Stark B, Resnitzky R, Jeison M, et al. A distinct subtype of M4/M5 acute myeloblastic leukemia (AML) associated with t(8;16)(p11;p13) in a patient with the variant t(8;19)(p11;q13)—case report and review of the literature. *Leuk Res*. 1995;19:367–379.
 36. Velloso ERP, Mecucci C, Michaux L, et al. Translocation t(8;16)(p11;p13) in acute non-lymphocytic leukemia: report on two new cases and review of the literature. *Leuk Lymphoma*. 1996;21:137–142.
 37. Jacobson S, Pillus L. Modifying chromatin and concepts of cancer. *Curr Opin Genet Dev*. 1999;9:175–184.
 38. Odom LF, Lampkin BC, Tannous R, et al. Acute monoblastic leukemia. A unique subtype—a review from the children’s cancer study group. *Leuk Res*. 1990;14:1–10.
 39. Osada S, Horibe K, Oiwa K. A case of infantile acute monocytic leukemia caused by vertical transmission of the mother’s leukemic cells. *Cancer*. 1990;65:1146–1149.
 40. Dinulos JG, Hawkins DS, Clark BS, et al. Spontaneous remission of congenital leukemia. *J Pediatr*. 1997;131:300–303.
 41. Fernandez MC, Weiss B, Atwater S, et al. Congenital leukemia successful treatment of a newborn with t(5;11)(q31;q33). *J Pediatr Hematol Oncol*. 1999;21:152–157.
 42. Weil M, Jacuillar C, Tobelem G. Therapy of acute monoblastic leukemia. *Haematol Blood Transf*. 1981;27:189–194.
 43. Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
 44. Case 50–1990. Case records of the Massachusetts General Hospital. *N Engl J Med*. 1990;323:1689–1697.

CASE 12

Acute Erythroid Leukemia

CASE HISTORY

A 69-year-old man was admitted to the hospital for evaluation of anemia and thrombocytopenia. One week prior to admission, the patient started to have low-grade fever, nausea, malaise, and nonbloody diarrhea. He also had several episodes of epistaxis during the past week. The blood work done in the physician’s laboratory revealed a hematocrit of 20% and platelets of 30,000/mL, and he was referred to our hospital for further evaluation. His routine hematology workup 3 months before admission was essentially normal except for a hematocrit of 34%.

On admission, the patient looked pale, but no petechiae or ecchymosis was found on the skin. Physical examination revealed no hepatosplenomegaly or lymphadenopathy. Laboratory data showed normal serum iron, lactate dehydrogenase, and fibrinogen. D-dimer, fibrinogen split products, and direct Coomb test were negative.

A bone marrow aspirate showed 87.7% normoblasts including 55.7% pronormoblasts. There were 10.3% myeloid cells, which included no myeloblasts. The pronormoblasts were pleomorphic with multiple intraplasmic vacuoles that were positive for periodic acid-Schiff (PAS)

stain. The maturing normoblasts revealed megaloblastoid changes and nuclear dysplasia.

A diagnosis of acute erythroid leukemia (AEL) was made. The patient’s family was informed of the poor prognosis in association with this disease. The patient refused to receive blood products, and the family opted to go home for home hospice care.

FLOW CYTOMETRY FINDINGS

The bone marrow aspirate revealed 3% myeloperoxidase (MPO), 25% CD13-CD33, 0% CD13-CD33/CD7, 2% CD14, 5% HLA-DR, 65% glycophorin A, 3% CD34 and 20% CD117 (Fig. 6.12.1).

CYTOGENETIC FINDINGS

Cytogenetic analysis of the bone marrow detected a complex abnormal karyotype. There was additional material on the short arm of chromosomes 7 and 21 and on the long arm of chromosome 11. A deletion of 5q and 7q and loss of chromosome 16 were also present.

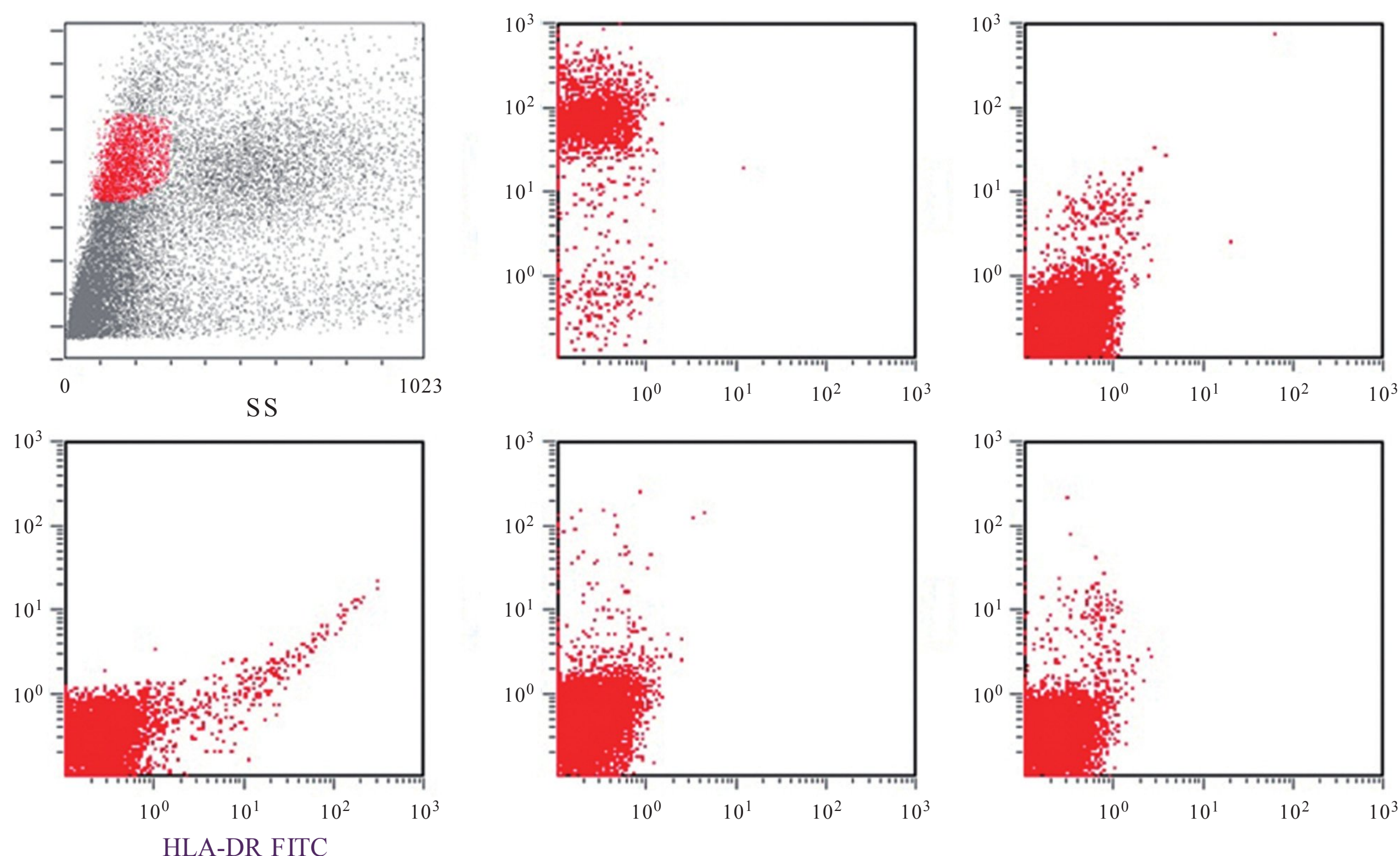


FIGURE 6.12.1 Flow cytometric analysis using the forward scatter (FS) versus side scatter (SS) plot, as the normoblasts are CD45 dim or negative. The histograms show positive glycophorin A reaction, but negative reactions for HLA-DR and CD34. Low percentages of CD117 and CD13-CD33 are detected. FITC, fluorescein isothiocyanate; RD1, rhodamine; PE, phycoerythrin.

DISCUSSION

AEL (AML-M6) is a rare disease, accounting for 4% to 5% of all acute myeloid leukemia (AML). Giovanni di Guglielmo was the first one to recognize this entity (1). In 1917, he described a syndrome with a mixed population of immature erythroid and myeloid cells (later referred to as di Guglielmo syndrome), and in 1926 he reported a disease with pure immature erythroid proliferation (later referred to as di Guglielmo disease) (2). However, this concept of subdivision of erythroleukemia did not draw attention in the field of hematology until recently. The French-American-British (FAB) criteria for M6 are the presence of at least 50% normoblasts (erythroblasts) among the total number of nucleated cells and 30% type I and type II blasts among the nonerythroid population in the bone marrow (Fig 6.12.2) (3). According to this classification, any case with <30% myeloblasts should be included in the myelodysplastic syndrome (MDS). However, Kowal-Vern et al. (4) recognized the prognostic significance of the immature erythroid components; cases with an increased pronormoblast to myeloblast ratio have worse prognosis than those with a higher myeloblast count (4). They suggested the subdivision of M6 into M6a (the original M6) and M6b (pure erythroid leukemia). The same concept

was presented by Garand et al. (5) and Hasserjian et al. (6); these groups designated the pure erythroid leukemia as AML-M6 variant. Mazzella et al. (7) further subdivided AMA-M6 into M6a, M6b, and M6c (7). This group defined M6c cases as those with both myeloblasts and pronormoblasts >30% of the nucleated cells.

In the 2001 World Health Organization (WHO) classification, the original erythroleukemia is designated erythroleukemia (erythroid/myeloid), and the requirement for the myeloblast count in the nonerythroid population has been reduced to $\geq 20\%$ (8–10). AML-M6b is designated as pure erythroid leukemia, which requires >80% of immature erythroid cells without a significant myeloblastic component in the bone marrow (Fig 6.12.3). The 2008 WHO classification retains the subdivisions of AEL, but moves AEL cases with more than 20% myeloblasts of the total bone marrow cells into a new entity designated “acute myeloid leukemia (AML) with myelodysplasia-related changes” (11,12).

Morphology and Cytochemistry

The characteristic morphologic features of AEL are the predominance of atypical erythroid precursors of all maturation stages and the presence of erythrodysplasia in the bone marrow. As mentioned before, the percentages of the erythroid precursors and myeloblasts distinguish

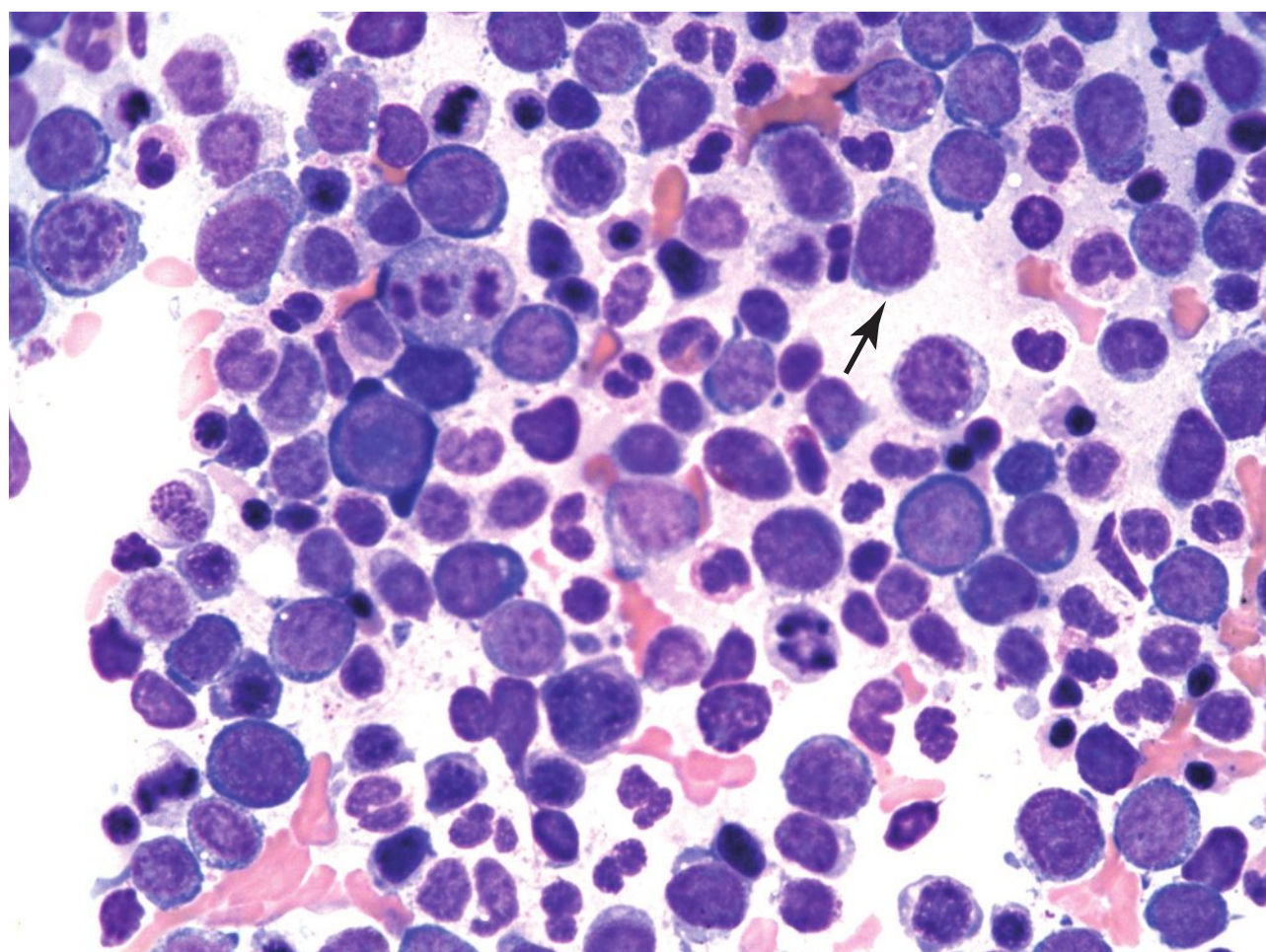


FIGURE 6.12.2 Bone marrow aspirate from a case of erythroleukemia (erythroid/myeloid) shows mainly pronormoblasts with a few myeloblasts (arrow). Wright–Giemsa, 60× magnification.

erythroleukemia (erythroid/myeloid) (M6a) from pure erythroid leukemia (M6b). However, a recent study from the M.D. Anderson Cancer Center showed that 8 of 13 M6b cases had <80% immature erythroblasts (13). It is suggested by this study that the WHO threshold for erythroblasts is arbitrarily high; therefore, if other parameters are typical for M6b, the erythroid element is not necessarily above 80% of all bone marrow cells. In the peripheral blood, anisopoikilocytosis, macrocytosis, schistocytes, and nucleated erythrocytes may be present, but blasts are seldom encountered.

The erythroid precursors are mainly composed of pronormoblasts and basophilic normoblasts in M6a, but they

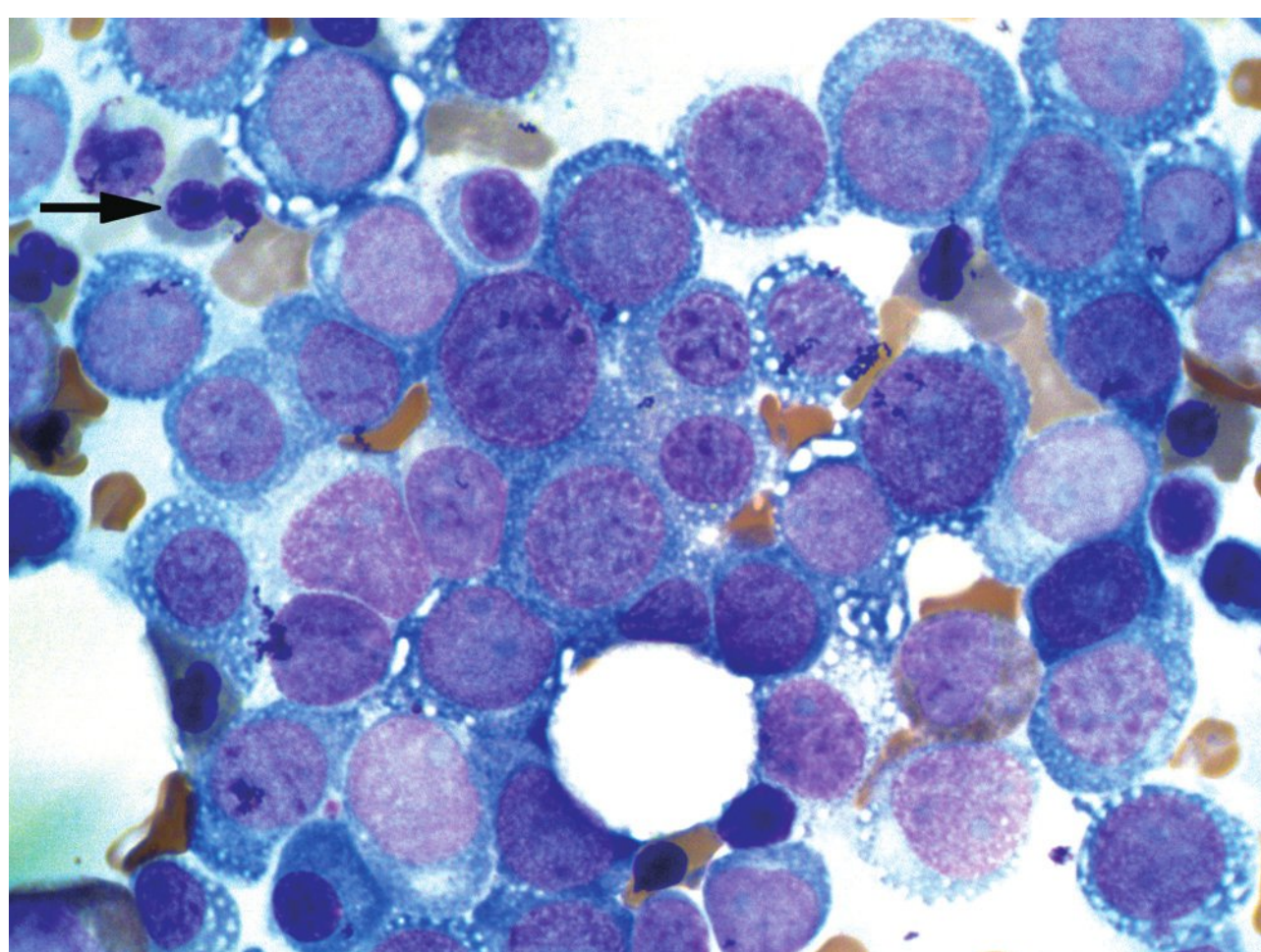


FIGURE 6.12.3 Bone marrow aspirate from a case of pure erythroid leukemia reveals predominantly pronormoblasts with other stages of normoblasts. Myeloblasts are absent. Note cytoplasmic vacuolation in pronormoblasts and a few dysplastic nucleated erythrocytes (arrow). Wright–Giemsa, 100× magnification.

are usually undifferentiated in M6b; cytochemistry and immunochemistry are often required for identification. The pronormoblasts are of large size with regular cell borders. The cytoplasm is deeply basophilic and devoid of granules. The nucleus is perfectly round with a delicate chromatin pattern, sometimes referred to as a sieve-like pattern. The leukemic pronormoblasts and basophilic normoblasts are highly pleomorphic, varying in size and shape. The nuclei can be polylobulated, multiple, fragmented, or extraordinarily large. One to a few prominent nucleoli are usually present. The cytoplasm is characterized by multiple vacuolation and lack of hemoglobinization.

The erythrodysplastic features include megaloblastoid/megaloblastic changes, nuclear budding, nuclear bridging, and other irregular configurations of the nucleus. The frequent presence of erythrodysplasia in AEL may be because most cases of AEL evolve through a myelodysplastic phase (14). AEL is frequently associated with a history of MDS, chemotherapy, or exposure to toxin or alcohol (7,15).

The myeloblasts in M6a are not different from those seen in M1 and M2. Auer rods can be found in occasional cases. Dysplastic features are seldom seen in myeloid and megakaryocytic lineages. If a suspicious M6a case shows $\geq 50\%$ dysplastic myeloid or megakaryocytic cells, it was classified as AML with multilineage dysplasia in the 2001 WHO classification (9,10). If in addition to multilineage dysplasia the myeloblasts are more than 20% of the total bone marrow cells, those cases should be diagnosed as AML with myelodysplasia-related changes (12). On the other hand, if myeloblasts is <20% in the nonerythroid population, even >50% normoblasts are present, it should be diagnosed as MDS rather than AEL (11,16). The distinction between M6a and refractory anemia with excess blasts is sometimes difficult, because when there is marked erythroid hyperplasia in MDS, the myeloblast count in the small population of nonerythroid nucleated cells may easily reach the 20% cutoff required for the diagnosis of M6a (17).

However, a recent study suggests that AEL is in the continuum of MDS and AML with erythroid hyperplasia, but these entities are arbitrarily divided by the blast counts (16). This group considers that the karyotype rather than an arbitrary blast cutoff represents the most important prognostic factor. In cases suspicious for M6b, vitamin B12 and folate deficiency and erythropoietin therapy should always be excluded before a diagnosis is made (2,9).

Cytochemical stain is helpful in substantiating the diagnosis of M6. PAS is normally negative in nucleated red blood cells. However, it is often positive in M6 cases showing coarse granules (block pattern) in pronormoblasts and basophilic normoblasts, and diffuse cytoplasmic staining in polychromatophilic and orthochromic normoblasts (Fig 6.12.4). A negative PAS stain, however, does not exclude the diagnosis of M6. The MPO, Sudan black B, and chloroacetate esterase stains are negative for normoblasts; if they are positive in M6 cases, the cells that pick up the stains are myeloblasts (Fig 6.12.5) (18). Some early normoblasts may show weak focal α -naphthyl butyrate esterase stain, which is, however, not helpful in the diagnosis. The Prussian blue stain for iron is helpful to detect ringed sideroblasts, which are seen more frequently in M6b than in M6a cases (2).

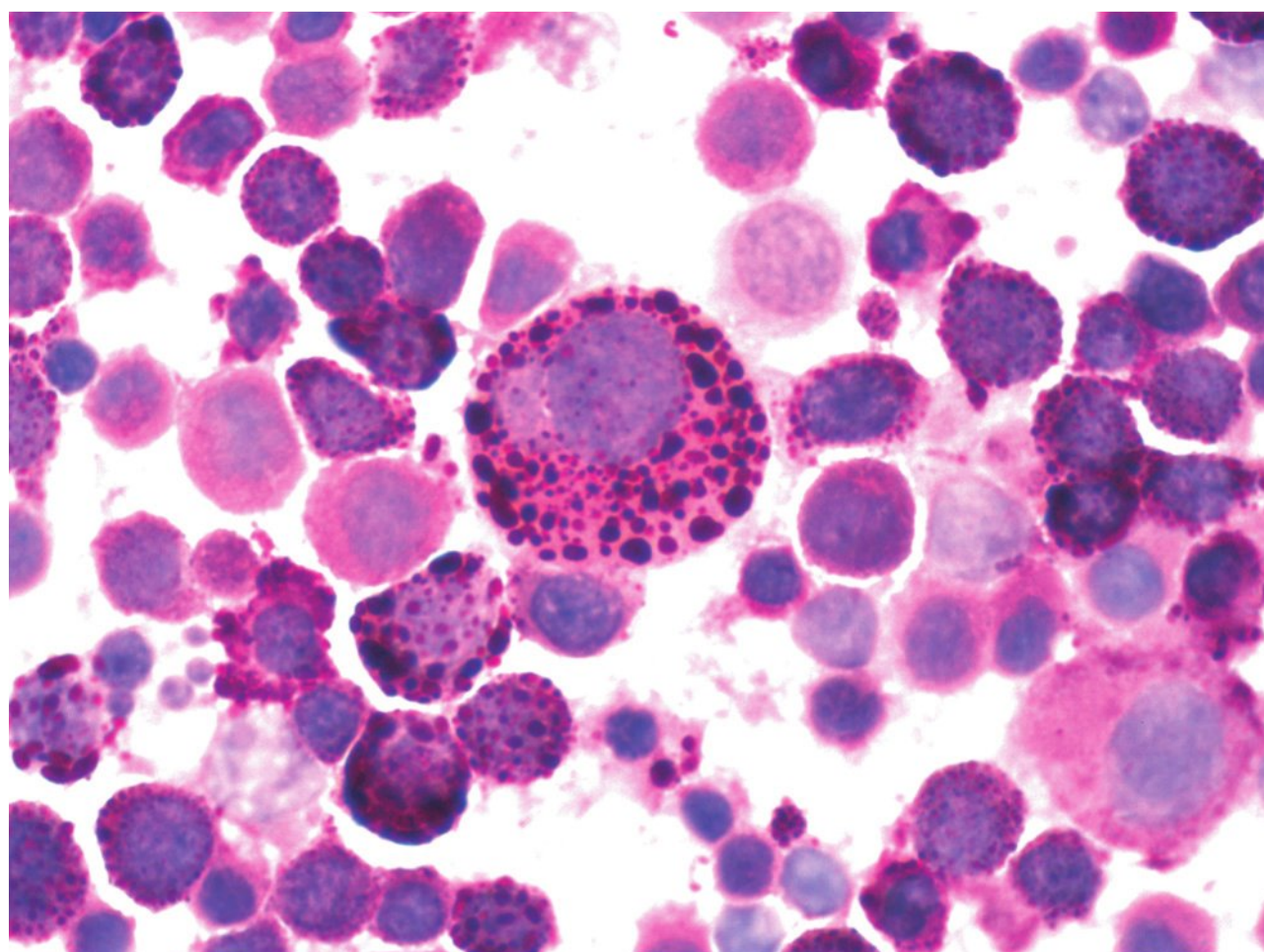


FIGURE 6.12.4 Bone marrow aspirate from a case of pure erythroid leukemia shows coarse PAS-positive granules in the cytoplasm of pronormoblasts and basophilic normoblasts, but diffuse staining in polychromatophilic and orthochromatic normoblasts. PAS, 100× magnification.

In the current case, the bone marrow contained 87% erythroid precursors including 55% pronormoblasts without the presence of myeloblasts. The pronormoblasts were pleomorphic, and the cytoplasm was vacuolated. The normoblasts other than the pronormoblasts showed marked nuclear dysplasia. The bone marrow biopsy was hypercellular with extensive erythroblastic infiltration (Figs. 6.12.6 and 6.12.7). The cytochemical stain demonstrated strong cytoplasmic PAS staining with a block pattern. The flow cytometry revealed 44% glycophorin A. With all this laboratory information, a definitive diagnosis of pure erythroid leukemia (M6b) was established.

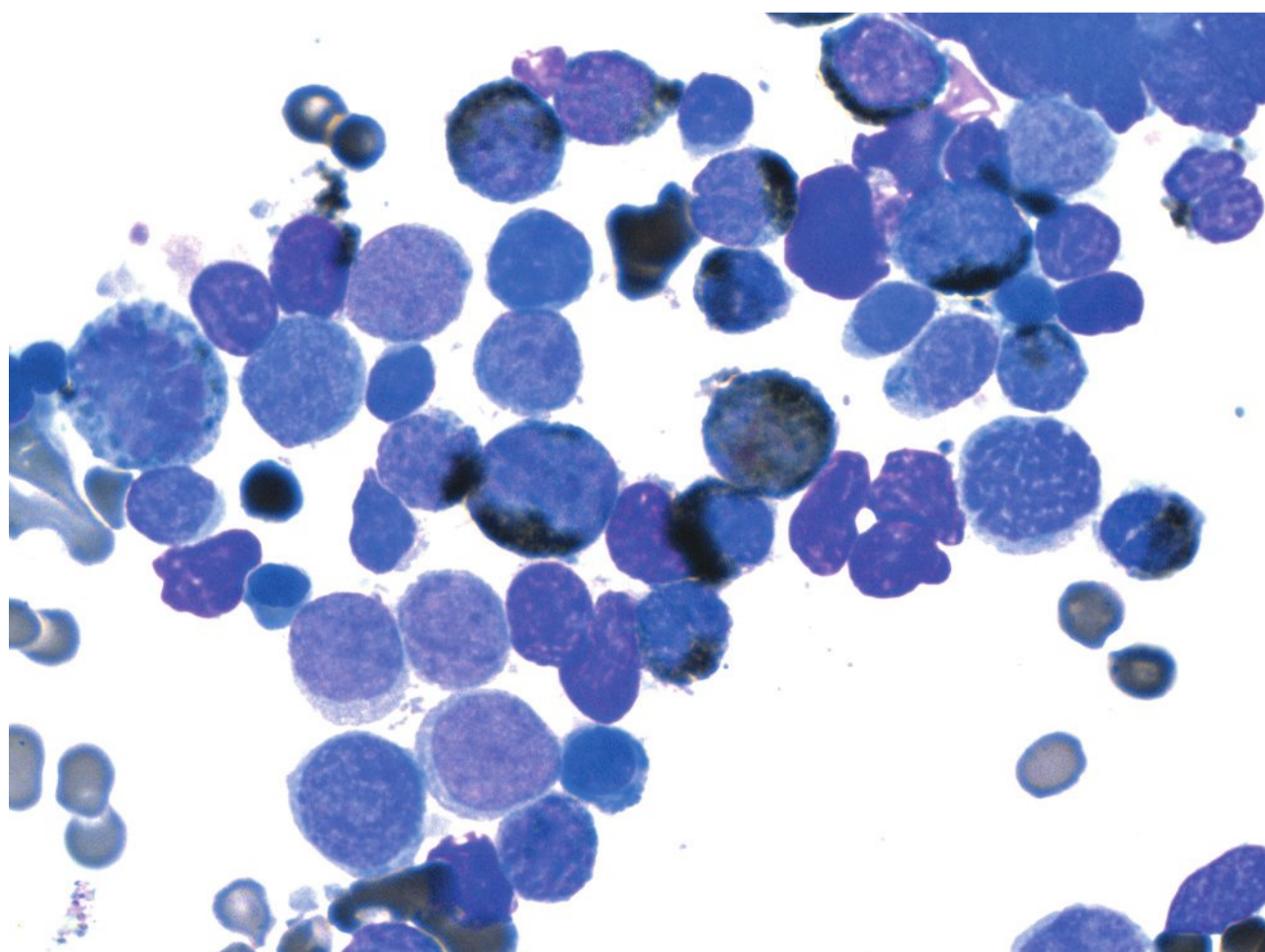


FIGURE 6.12.5 Bone marrow aspirate from a case of M6a reveals MPO staining in the myelocytic series including myeloblasts, but the normoblasts are negative. MPO, 100× magnification.

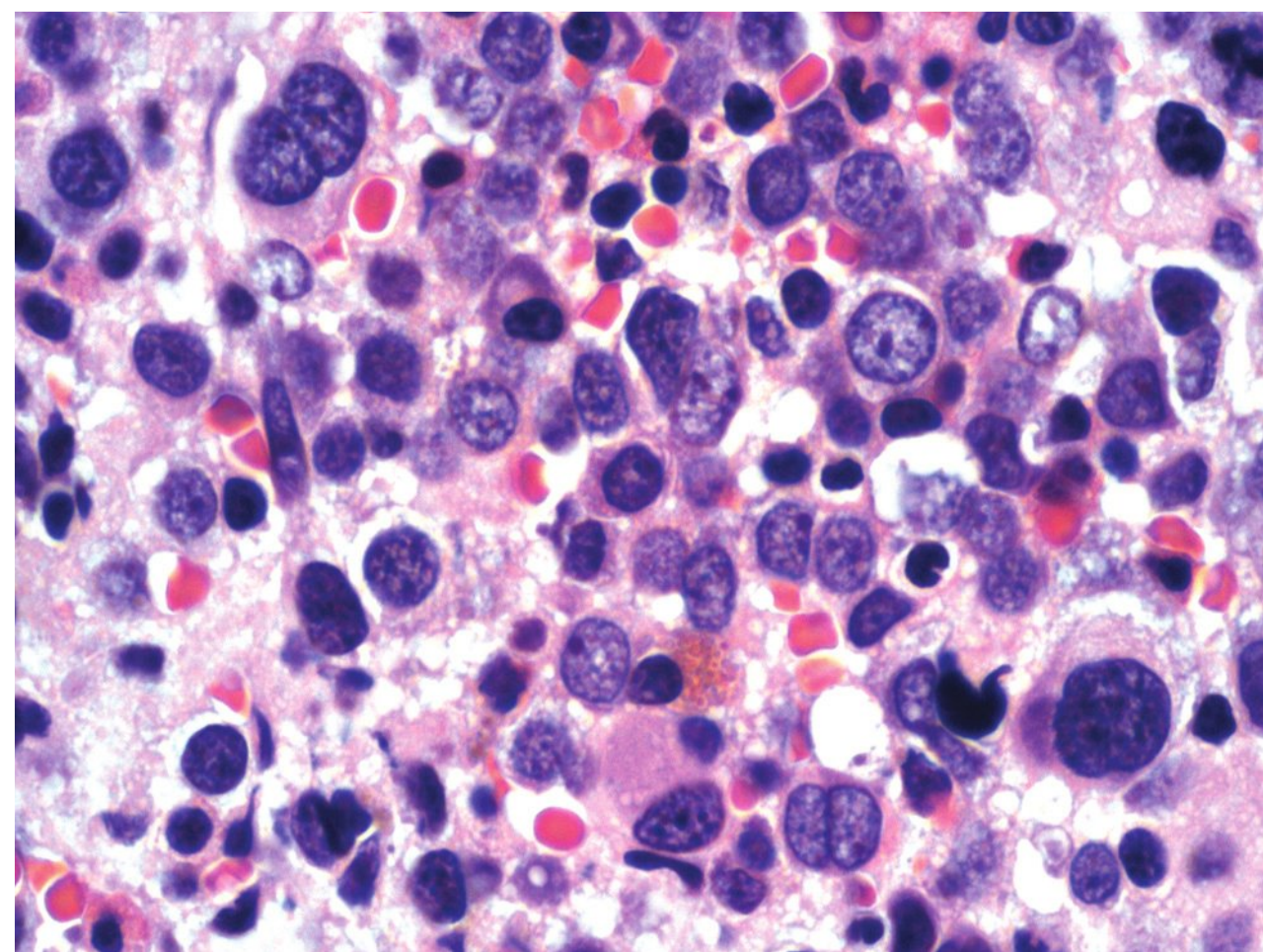


FIGURE 6.12.6 Bone marrow core biopsy from a case of pure erythroid leukemia (M6b) shows a predominant pleomorphic erythroblastic population with a few giant cells and binucleated cells. Hematoxylin and eosin, 100× magnification.

Immunophenotype

In M6a cases, the erythroid precursors are usually recognizable. However, M6b cases often show primitive blasts, and the PAS stain can be negative or equivocal; therefore, immunophenotyping is frequently required for a final diagnosis.

Several antibodies can help to identify the erythroid series. Early antibodies include antihemoglobin and anti-carbonic anhydrase I antisera, which identify the normal components of erythrocytes, but these components may not be present in very immature cells (19,20). The mouse monoclonal antibody, FA6-152, is positive for erythrocyte burst-forming units, erythrocyte colony-forming units, pronormoblasts, and normoblasts, but it is also present in normal monocytes and megakaryocytes (21). A murine

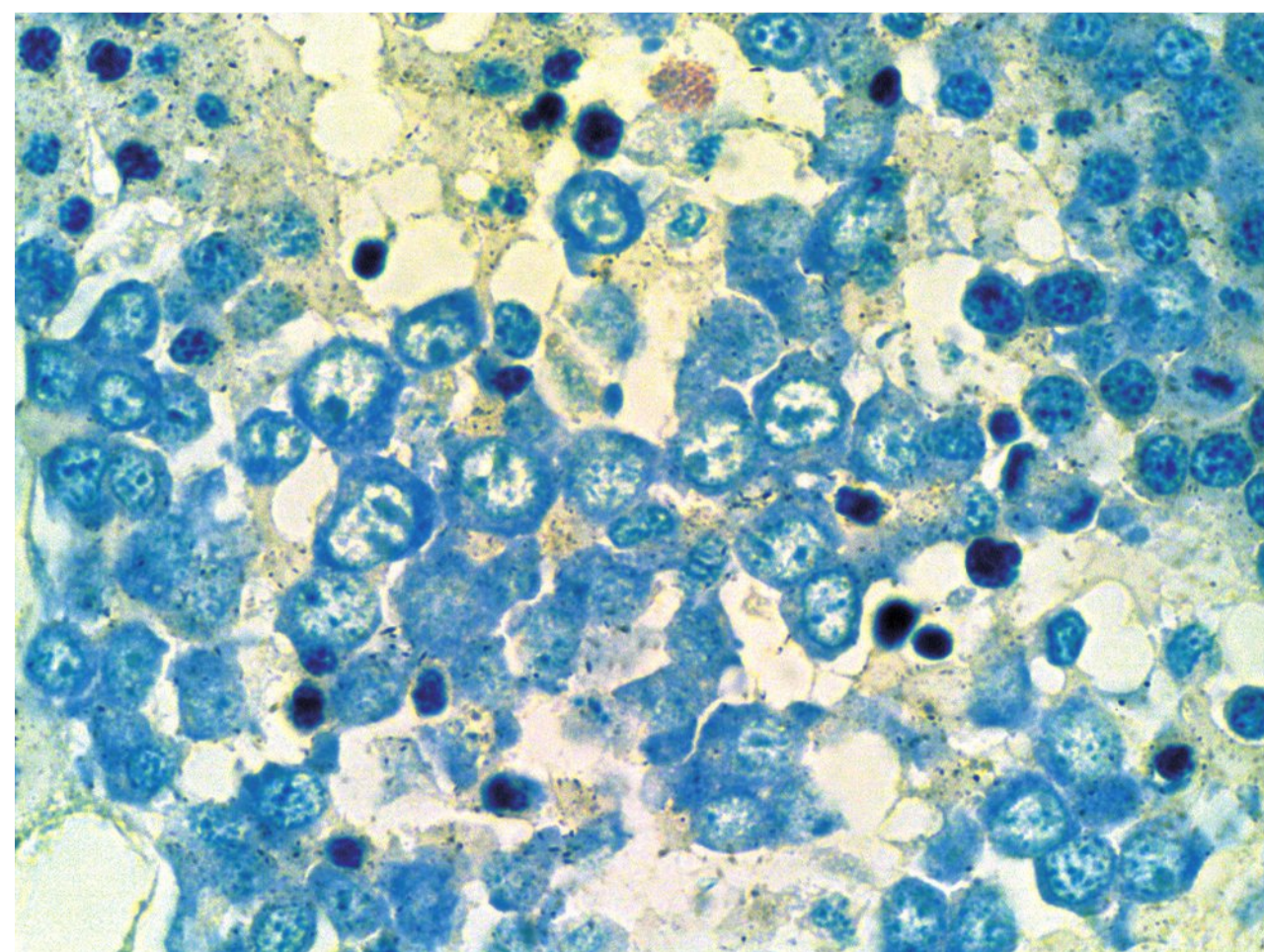


FIGURE 6.12.7 Pronormoblasts in a bone marrow biopsy readily recognizable in a Giemsa-stained preparation. Giemsa, 100× magnification.

monoclonal antibody developed at the Mayo Clinic, RC82.4, is reported to be specific and sensitive in detecting normal and leukemic erythroid cells without cross-reactivity to other cell lineages (20). However, the nature of the antigen with which RC-82.4 reacts is still not clear, and the antibody is not commercially available.

Gupta and Dhond (22) used a panel of monoclonal antibodies specific for different developmental stages (erythrocyte burst-forming units, erythrocyte colony-forming units, normoblasts, erythrocytes) and components (glycophorin A and H antigens) of erythroid cells and found that, in most cases of M6, the phenotype of the pronormoblasts was that of the intermediate stage of maturation (23). Transferrin receptor antibody (CD71) has also been used to detect mature and immature nucleated erythrocytes (22). Although CD71 has been used in the monoclonal antibody panel for the diagnosis of M6 (24), it is an activation antigen, presenting in various conditions, and thus not specific for M6. The erythroid precursors may express CD36, but this antigen is also present on megakaryocytes and monocytes (8).

Greaves et al. (25) found that 78% of 27 cases of M6 reacted with glycophorin-A antibody, but only 3% of 724 cases of nonerythroid leukemias had a positive reaction to that antibody. Because of its specificity and availability, glycophorin-A antibody has become the most widely used for the identification of erythroid cells and diagnosis of M6 (26). Glycophorin-A antibody has been used in flow cytometry and, more recently, in immunohistochemistry.

Using glucose-6-phosphate dehydrogenase isoenzyme analysis, it has been found that M6 is a clonal disorder arising from a multipotent stem cell (27). The human erythroleukemia cell line expresses surface antigens of the erythroid, macrophage, and megakaryocyte lineages (28). This cell line can be induced by different agents into full expression of erythroid, macrophage, or megakaryocyte phenotypes (28). Therefore, it is not surprising to see that leukemic cells in M6 cases may react to myeloid antigens (CD11b, CD13, CD15, and CD33) and platelet antigen (CD41) (2,14,22). However, the reactions to these antigens are not consistent, and the results are sometimes due to the coexistence of myeloblasts and increased megakaryocytes in the bone marrow; thus, myelomonocytic or megakaryocytic antigens should not be depended on for the diagnosis of M6.

CD34 was present in 26% of M6a and 26.7% of M6b cases in one study (7). However, the percentage of CD34 was proportional to that of myeloblasts rather than pronormoblasts. The expression of CD117 (c-kit) is associated with myeloblasts and some relatively mature normoblasts (9,11).

Comparison of Flow Cytometry and Immunohistochemistry

Immunohistochemical stains can demonstrate erythroid precursors with glycophorin-A (Fig 6.12.8) and hemoglobin A antibodies (Fig 6.12.9) (9). Myeloblasts can be detected with CD34 and CD117 together with other myeloid antibodies (e.g., MPO, CD33) (Fig 6.12.10). CD117 may also be demonstrated in normoblasts (Fig 6.12.11). In contrast,

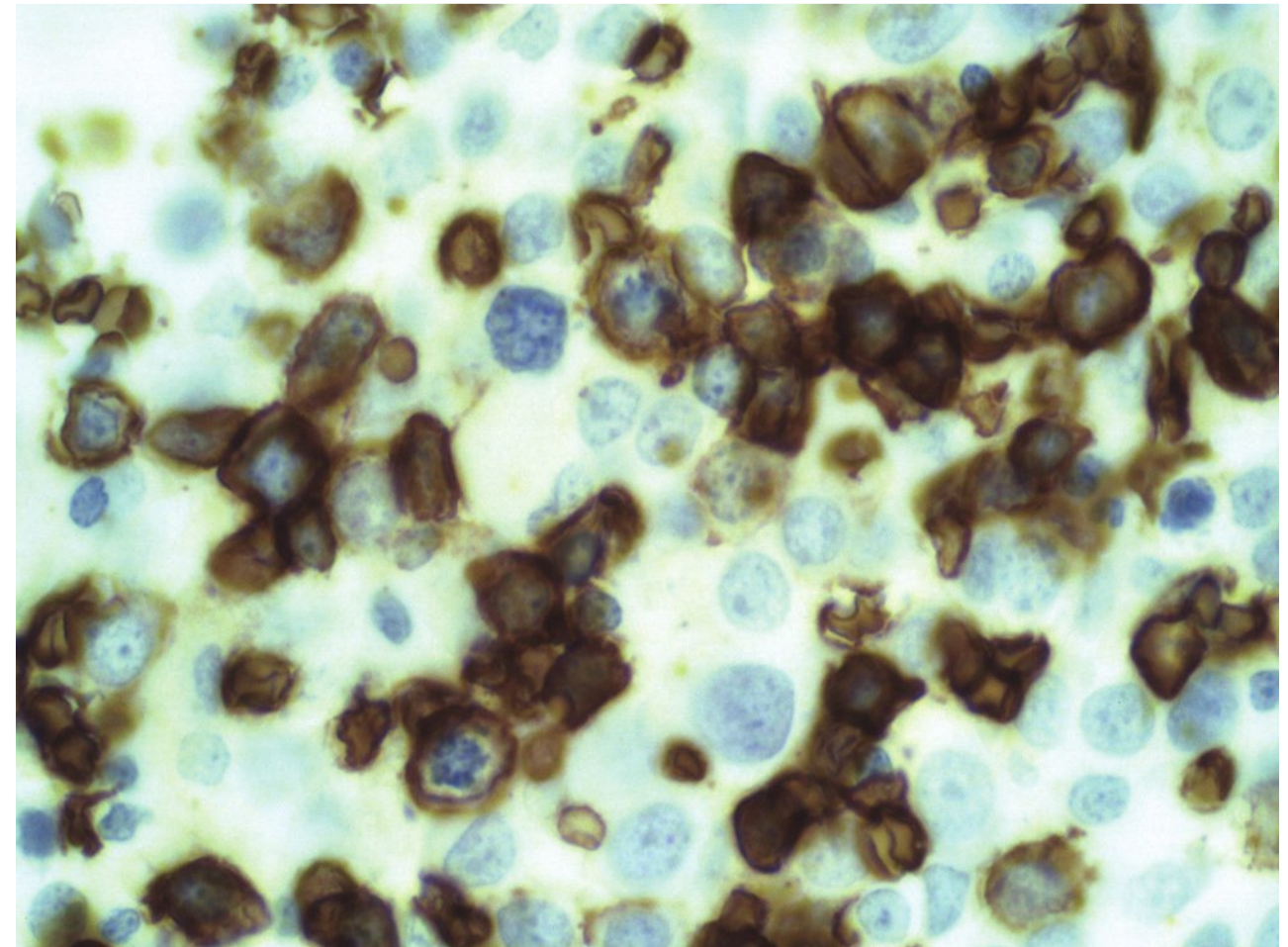


FIGURE 6.12.8 Bone marrow biopsy from an M6b case shows that most normoblasts are positive for glycophorin A. Immunoperoxidase, 100× magnification.

glycophorin A is the only antibody useful for the diagnosis of M6 by flow cytometry. The increase of glycophorin A as detected by flow cytometry can be seen in cases with marked erythroid hyperplasia; thus, it is not always diagnostic.

Molecular Genetics

The percentage of aneuploidy is particularly high (63%) in M6 (29). Most cases show complex karyotypes with multiple structural abnormalities (9). Monosomy or long-arm deletions of chromosome 5 and/or 7 occur most frequently in various studies (7,11,13,14,16,29). However, a recent study of 20 AEL cases found 15 patients with diploid cytogenetics and only 5 patients with complex karyotypes (30). The karyotype demonstrated in the current case is characteristic. The two chromosomal aberrations, that is, -5/del(5q) and -7/del(7q), are most frequently associated with MDSs, which may precede the development of M6.

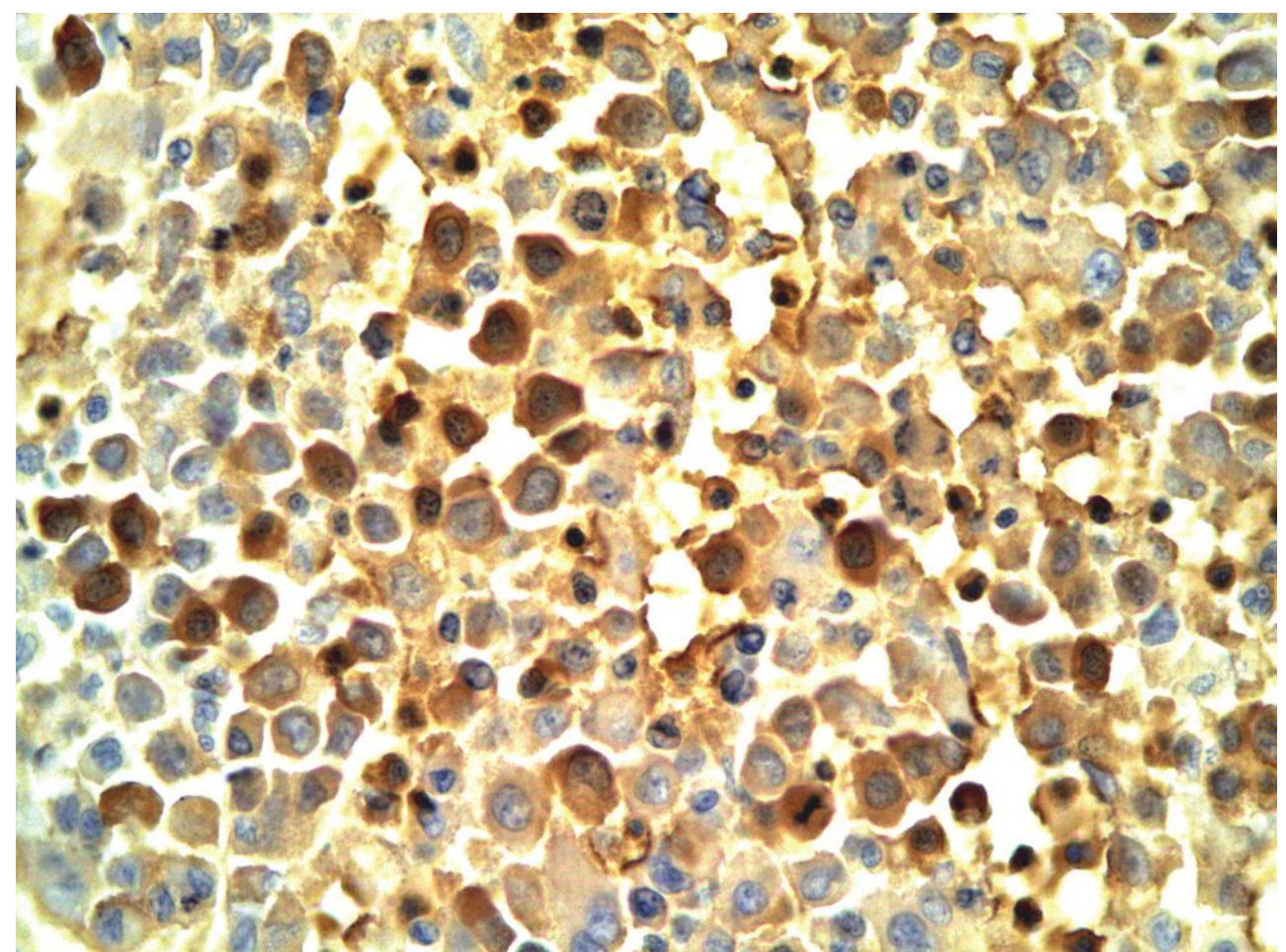


FIGURE 6.12.9 Bone marrow biopsy from an M6b case shows hemoglobin A staining of erythroid cells. 60× magnification.

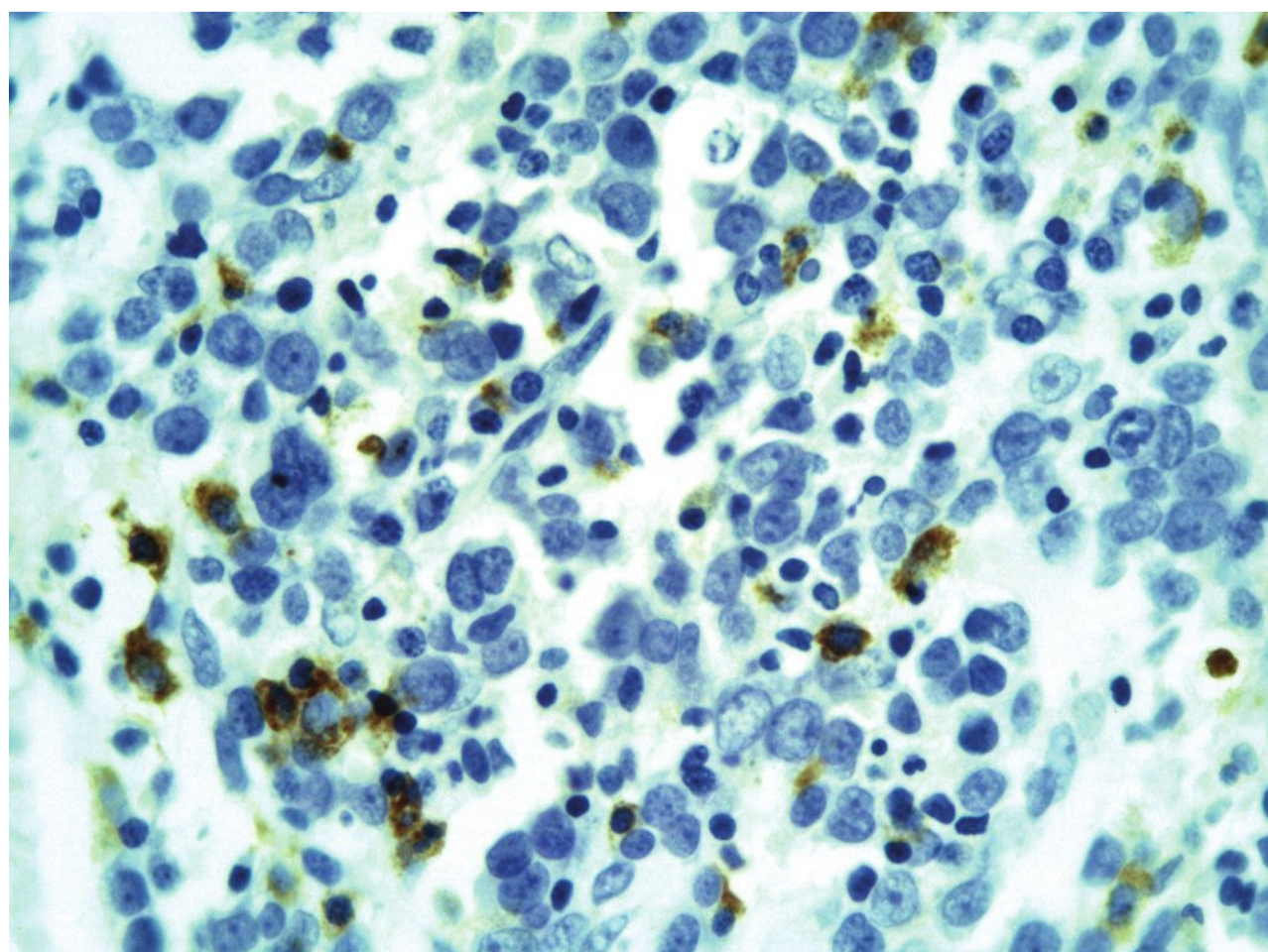


FIGURE 6.12.10 Bone marrow biopsy from an M6b case reveals no immunoperoxidase-positive blasts. Immunoperoxidase, 60× magnification.

Cuneo et al. (14) divide M6 cases into two groups: Those with three or more cytogenetic abnormalities are designated major karyotype aberrations (MAKA), and those with a single abnormality are designated minor karyotype aberrations (MIKA). The MAKA group is always associated with increased immature erythroid precursors, and the MIKA group with preserved maturation of erythroid cells. The MAKA group has lower hemoglobin levels, lower complete remission rates, and shorter survival than the MIKA group (14). Apparently, this cytogenetic–cytopathologic classification is of prognostic significance. Mazzella et al. (7) found that 8 of 16 M6a cases had a normal karyotype, 4 had MIKA, and 4 had MAKA, whereas 10 of 11 M6b cases had MAKA.

In a case of M6 with monosomy 7, fluorescence in situ hybridization identified the same clonal abnormality in

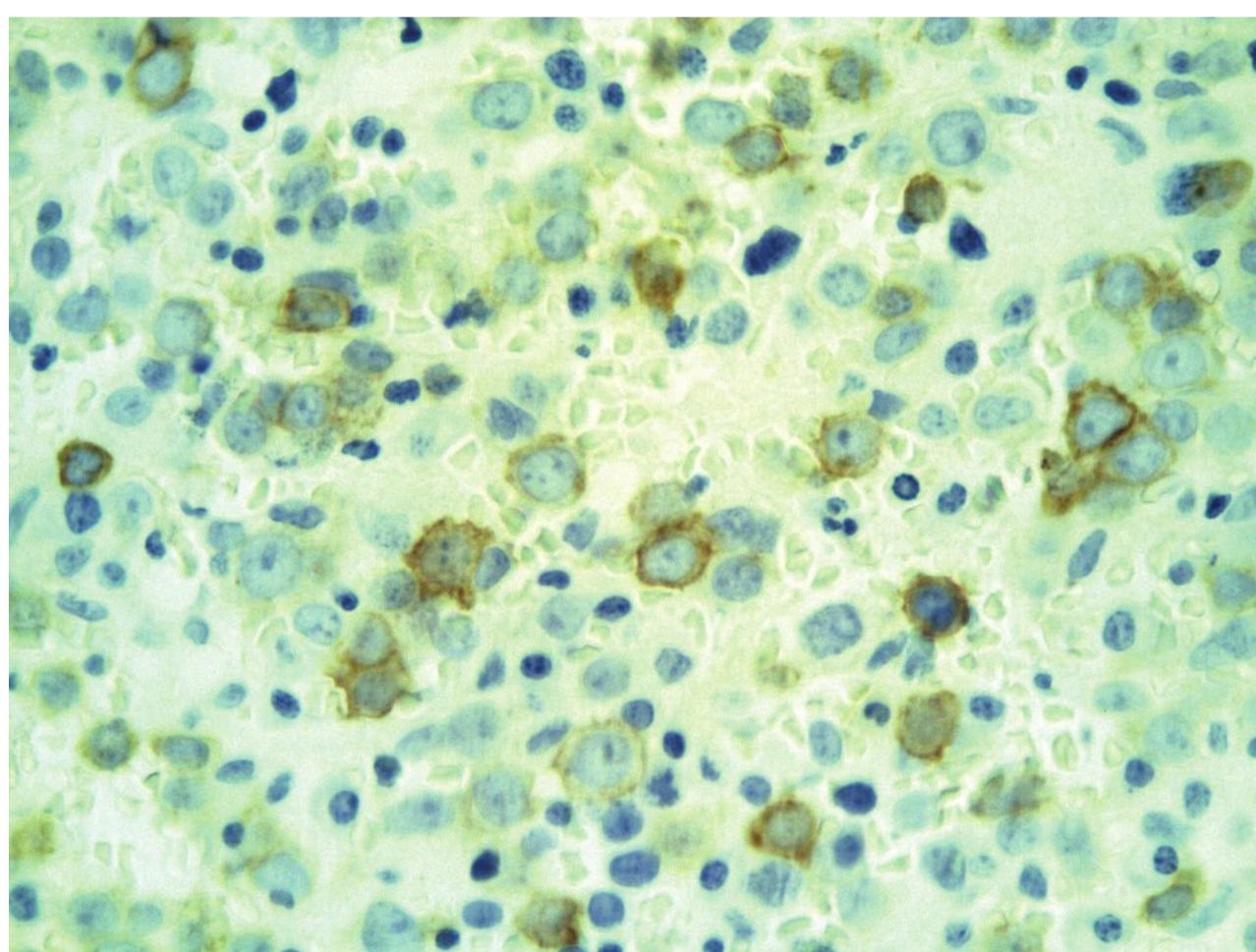


FIGURE 6.12.11 Bone marrow biopsy from an M6b case shows a few CD117-positive normoblasts. Immunoperoxidase, 60× magnification.

both erythroid and myeloid lineage, and the normal erythroid population coexisted with the leukemic erythroid population (31). These findings substantiate the theory that M6 is a clonal disorder arising from a multipotent stem cell. Multidrug resistance gene expression and p53 gene mutation were demonstrated in all subtypes of M6, but more frequently in M6b and M6c than in M6a (2).

The salient features for laboratory diagnosis of M6 are summarized in Table 6.12.1.

Clinical Manifestations

Clinically, the presenting symptoms are usually associated with severe anemia. M6 cases have the lowest hemoglobin level and leukocyte and platelet counts, compared with other subtypes of AML, partly because M6 is often preceded by a MDS (32). Although nucleated red blood cells are frequently present, pronormoblasts are seldom detected in the peripheral blood (15). Hepatosplenomegaly and lymphadenopathy occur in <25% of patients (31).

M6 is often seen in elderly persons, but a subset of younger patients with better clinical outcome has been found in one study (15). Congenital, familial erythroleukemia or erythroleukemia of infancy and childhood have been occasionally reported (33–36). M6 may also be presented as a blastic crisis of a myeloproliferative disorder, such as chronic myelogenous leukemia or polycythemia vera (2,5,37).

In a study by Mazzella et al. (7), the increase of proliferation markers (proliferating cell nuclear antigen and Ki-67) correlated positively with pronormoblast count and multiple cytogenetic abnormalities and inversely with survival and erythroid lineage maturation. They also found

TABLE 6.12.1

Salient Features for Laboratory Diagnosis of M6

1. M6a: Presence of $\geq 50\%$ normoblasts among all nucleated cells and $\geq 20\%$ myeloblasts among non-erythroid cells in the bone marrow. If myeloblasts are $\geq 20\%$ of total bone marrow cells, AML with myelodysplasia-related changes should be considered
2. M6b: Presence of $\geq 80\%$ immature erythroid precursors with no significant myeloblast component
3. Positive PAS staining in mature and immature nucleated erythroid cells
4. Glycophorin-A and hemoglobin-A staining by immunohistochemistry and glycophorin-A positivity by flow cytometry. CD117 is positive in some normoblasts.
5. Myeloblasts in M6a cases show myeloid markers (e.g., CD13, CD33, MPO) and immature cell markers (CD34 and CD117).
6. Complex abnormal karyotypes in most cases, with frequent -5/5q- and -7/7q-

that patients with increased numbers of myeloblasts and few pronormoblasts had the best prognosis, whereas survival rapidly declined with decreasing myeloblast counts. Accordingly, the authors suggested that chemotherapy for M6 should be directed toward both myeloblasts and pronormoblasts to replace the current regimens that do not affect pronormoblasts. Another study found that the overall survival was only related to cytogenetic risk group, but not blast count or morphologic dysplasia (16).

Therapeutic effects also depend on disease status at presentation. In 19 de novo cases of M6, the remission rate after induction chemotherapy was 95% and the relapse rate was 35%, whereas 8 cases with secondary M6 had a remission rate of 57% and a relapse rate of 75% (38). The reported median survival for M6 varies from 4 to 14 months (39). With autologous hematopoietic stem cell transplantation, the leukemia-free survival (LFS) was $26\% \pm 5\%$ at 5 years, whereas the LFS was $57\% \pm 5\%$ for allogeneic hematopoietic stem cell transplantation (39). A recent study found that AML-M6 did not impart by itself a worse outcome, only the association with MDS and poor-risk karyotype that conveyed a poor prognosis (13). Another study showed that AEL had the best overall survival as compared to AML with myelodysplasia-related changes, therapy-related AML, and refractory anemia with excess blasts (30).

REFERENCES

- Bain BJ. Historical review: Di Guglielmo and his syndromes. *Br J Haematol*. 2003;120:939–943.
- Mazzella FM, Alvares C, Kowal-Vern A, et al. The acute erythroleukemias. *Clin Lab Med*. 2000;20:119–137.
- Bennett JM, Catovsky D, Daniel MT, et al. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:626–629.
- Kowal-Vern A, Cotelingam J, Schumacher HR. The prognostic significance of proerythroblasts in acute erythroleukemia. *Am J Clin Pathol*. 1992;98:34–40.
- Garand R, Duchayne E, Blanchard D, et al. Minimally differentiated erythroleukemia (AML M6 ‘variant’): a rare subset of AML distinct from AML M6. *Groupe Francais d’Hematologie cellulaire. Br J Haematol*. 1995;90:868–875.
- Hasserjian RP, Howard J, Wood A, et al. Acute erythremic myelosis (true erythroleukaemia): a variant of AML FAB-M6. *J Clin Pathol*. 2001;54:205–209.
- Mazzella FM, Kowal-Vern A, Shrit A, et al. Acute erythroleukemia: evaluation of 48 cases with reference to classification, cell proliferation, cytogenetic, and prognosis. *Am J Clin Pathol*. 1998;110:590–598.
- Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
- Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia not otherwise categorized. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:91–105.
- Vardiman JW, Harris NL, Brunning RD. The World Health Organization (WHO) classification of the myeloid neoplasms. *Blood*. 2002;100:2292–2302.
- Arber DA, Brunning RD, Orazi A, et al. Acute erythroid leukemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:134–136.
- Arber DA, Brunning RD, Orazi A, et al. Acute myeloid leukemia with myelodysplasia-related changes. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:124–126.
- Santos FPS, Faderl S, Garcia-Manero G, et al. Adult acute erythroleukemia: an analysis of 91 patients treated at a single institution. *Leukemia*. 2009;23:2275–2280.
- Cuneo A, Van Orshoven A, Michaux JL, et al. Morphologic, immunologic and cytogenetic studies in erythroleukemia: evidence for multilineage involvement and identification of two distinct cytogenetic-clinicopathological types. *Br J Haematol*. 1990;75:346–354.
- Arkinson J, Hrisinko MA, Weil SC. Erythroleukemia: a review of 15 cases meeting 1985 FAB criteria and survey of the literature. *Blood Rev*. 1992;6:204–214.
- Hasserjian RP, Zuo Z, Garcia C, et al. Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. *Blood*. 2010;115:1985–1992.
- Selby DM, Valdez R, Schnitzer B, et al. Diagnostic criteria for acute erythroleukemia. *Blood*. 2003;101:2895–2896.
- Li CY, Yam LT, Sun T. *Modern Modalities for the Diagnosis of Hematologic Neoplasms*. New York: Igaku-Shoin; 1996:13.
- Villeval JL, Cramer P, Lemonine F, et al. Phenotype of early erythroblastic leukemia. *Blood*. 1986;68:1167–1173.
- Solberg LA, Oles KJ, Kimlinger TK, et al. A new murine monoclonal antibody for the diagnosis of erythroleukemia. *Am J Clin Pathol*. 1990;93:387–390.
- Edelman P, Vinci G, Villeval JL, et al. A monoclonal antibody against an erythrocyte ontogenic antigen identifies fetal and adult progenitors. *Blood*. 1986;67:56–63.
- Gupta AS, Dhond SR. Phenotypic heterogeneity of erythroblasts in erythroblastic leukemia revealed by monoclonal antibodies. *Am J Hematol*. 1998;29:12–17.
- Yokochi T, Brice M, Rabinovith PS, et al. Monoclonal antibodies detecting antigenic determinants with restricted expression on erythroid cells from the erythroid committed progenitor levels to the mature erythroblast. *Blood*. 1984;63:1376–1484.
- Ngyyen AN, Milam JD, Johnson KA, et al. A relational database for diagnosis of hematopoietic neoplasms using immunophenotyping by flow cytometry. *Am J Clin Pathol*. 2000;113:95–106.
- Greaves MF, Sieff C, Edward PAW. Monoclonal anti-glycoprotein as a probe for erythroleukemias. *Blood*. 1983;61:645–651.
- Edward PAW. Monoclonal antibodies that bind to the human erythrocyte membrane glycoproteins glycophorin A and band 3. *Biochem Soc Trans*. 1980;8:334–336.
- Ferraris AM, Canepa L, Mareni C, et al. Re-expression of normal stem cells in erythroleukemia during remission. *Blood*. 1983;62:177–179.
- Long MW, Heffner CH, Williams JL, et al. Regulation of megakaryocyte phenotype in human erythroleukemia cells. *J Clin Invest*. 1990;85:1072–1084.
- Rowley JD, Alimena G, Garson DM, et al. A collaborative study of the relationship of the morphological type of acute nonlymphocytic leukemia with patient age and karyotype. *Blood*. 1982;59:1013–1022.
- Kasyan A, Medeiros LJ, Zuo Z, et al. Acute erythroid leukemia as defined in the World Health Organization

classification is a rare and pathogenetically heterogeneous disease. *Mod Pathol*. 2010;23(8):1113–1126.

31. Wong KF, Chu YC, Kwong YL. Abnormal erythropoiesis in erythroleukemia: a fluorescence in situ hybridization study. *Cancer Genet Cytogenet*. 1998;105:187–189.
32. Peterson BA, Levine EG. Uncommon subtypes of acute nonlymphocytic leukemia: clinical features and management of FAB M5, M6, and M7. *Semin Oncol*. 1987;14:425–434.
33. Allen RR, Wadsworth LD, Kalousek DK, et al. Congenital erythroleukemia: a case report with morphological, immunophenotypic and cytogenetic findings. *Am J Hematol*. 1989;31:114–121.
34. Hadjiyannakis A, Fletcher WA, Lebrun DP. Congenital erythroleukemia in a neonate with severe hypoxic ischemic encephalopathy. *Am J Perinatol*. 1998;30:395–401.
35. Novik Y, Marino P, Makower DF, et al. Familial erythroleukemia: a distinct clinical and genetic type of familial leukemias. *Leuk Lymphoma*. 1998;30:395–401.
36. Malkin D, Freedman MH. Childhood erythroleukemia: review of clinical and biological features. *Am J Pediatr Hematol Oncol*. 1989;11:348–359.
37. McFarlane R, Sun T. Detection of BCR/ABL fusion product in normoblasts in a case of chronic myelogenous leukemia. *Am J Surg Pathol*. 2004;28:1240–1244.
38. Killick S, Matutes E, Powles RL, et al. Acute erythroid leukemia (M6): outcome of bone marrow transplantation. *Leuk Lymphoma*. 1999;35:99–107.
39. Fouillard L, Labopin M, Gorin NC, et al. Hematopoietic stem cell transplantation for de novo erythroleukemia: a study of the European Group for Blood and Marrow Transplantation (EBMT). *Blood*. 2002;100:3135–3140.

CASE 13

Acute Megakaryoblastic Leukemia

CASE HISTORY

A 48-year-old man presented with fatigue and night sweats for 2 weeks. The patient was previously in good health and went to donate blood in the hospital, just to find out that he had a low leukocyte count. Since then he noticed low-grade fever and night sweats. Upon consulting his primary care physician, he was found to have pancytopenia. He further developed symptoms of dyspnea on exertion while walking up stairs or playing with his children. The patient was referred to a hematologist who admitted him to the hospital for further evaluation.

Physical examination on admission was unremarkable except for the presence of petechiae on his left and right anterior shins. He had no lymphadenopathy or hepatosplenomegaly. Laboratory examination of the peripheral blood showed a total leukocyte count of 1,900/mL, hematocrit of 14.6%, hemoglobin of 4.9 g/dL, and platelet count of 23,000/mL. The differential count revealed 71.1% lymphocytes, 24.3% neutrophils, and 2.3% monocytes, but no immature leukocytes were found.

After admission, a bone marrow biopsy was performed. It revealed 90% cellularity with many megakaryocytes of varying size and shape, which stained positive for CD42b. The bone marrow aspirate showed 52% blasts; many of them had cytoplasmic blebs.

A diagnosis of acute megakaryoblastic leukemia (AMKL; French-American-British [FAB] classification acute myeloid leukemia [AML]-M7) was established, and induction chemotherapy was started with ARA-C and daunomycin. During the course of treatment, the patient developed neutropenic fever and diarrhea associated with chemotherapy.

However, his condition was under control after antibiotic treatment. A repeat bone marrow biopsy showed complete remission, and he was discharged 1 month after admission.

Subsequently, the patient went through multiple cycles of consolidation and salvage therapy, but leukemia relapsed 2 months after the first admission. During this course, he had multiple episodes of neutropenic fever, pulmonary and hepatic aspergillosis, *Staphylococcus B* bacteremia, and *Clostridium difficile* colonization. Although the infections were treated successfully with various regimens of antibiotics, his leukemia became refractory to chemotherapy. Many blasts finally appeared in the peripheral blood, and the patient died 1 year after the initial diagnosis of M7.

FLOW CYTOMETRY FINDINGS

The bone marrow showed 0% myeloperoxidase (MPO), 91% CD13-CD33, 42% CD13-CD33/CD7, 82% CD41, 0% CD42, 75% CD61, 76% CD34, 0% terminal deoxynucleotidyl transferase, 5% glycophorin A, and 0% CD14 (Fig. 6.13.1).

CYTOCHEMICAL FINDINGS

The leukemic cells in the bone marrow were negative for MPO and α-naphthyl butyrate esterase but positive for periodic acid-Schiff (PAS) stains. The PAS stain showed a typical peripheral pattern with strong staining in the cytoplasmic blebs.

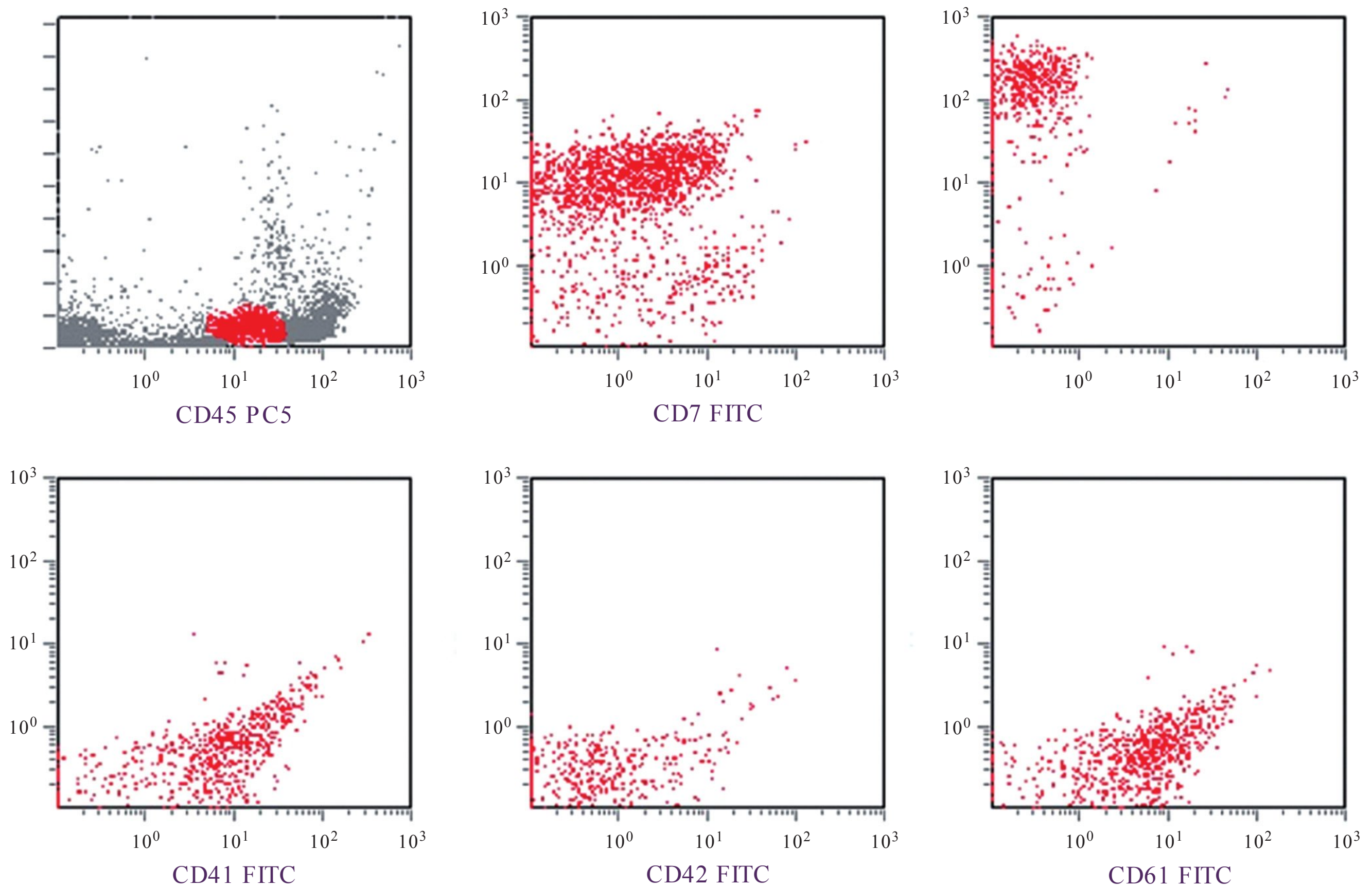


FIGURE 6.13.1 Flow cytometric analysis shows positive CD13-CD33, partially positive CD7, and positive CD34 reactions. The diagnostic feature is the expression of the immature megakaryocyte markers (CD41 and CD61) with low percentage or negative reaction of the mature megakaryocytic marker (CD42). SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; RD1, rhodamine; FITC, fluorescein isothiocyanate.

DISCUSSION

AMKL is a rare disease with a bimodal age distribution. The incidence of AMKL differs in various age groups. It accounts for 1% to 10% of AML in adults, 3.1% to 10% in childhood AML, and about 20% in AML of infants (1–4). The incidence of AMKL in children with Down syndrome (DS) is estimated to be approximately 500 times greater than that in children without this syndrome (5). There is evidence to indicate that AMKL in these various groups of patients may be biologically different, as they differ in cytogenetic profile and prognosis (6).

AMKL is classified as AML-M7 in the FAB system (7). Its diagnostic criterion is the presence of $\geq 30\%$ megakaryoblasts in the bone marrow. In the World Health Organization (WHO) classification, the requirement for the blast count is reduced to 20%, but more than one half of the blasts should be identified as of megakaryocytic lineage (8–10).

In contrast to criteria for other subtypes of AML, the FAB scheme requires the identification of megakaryocytic cells not only by morphology but also by either the platelet peroxidase reaction on electron microscopy or staining with monoclonal or polyclonal platelet specific antibodies. Because myelofibrosis or increased bone marrow reticulin

is a common finding in patients with AMKL, satisfactory bone marrow aspirate may be difficult to obtain and characteristic megakaryoblasts are difficult to find. In those cases, a diagnosis of AMKL is allowed on the estimation of the number of blasts in the bone marrow biopsy (7,9). Under this condition, unequivocal megakaryoblasts should be identified in the peripheral blood and/or bone marrow by immunologic techniques (7).

Morphology and Cytochemistry

The morphology of megakaryoblasts is highly polymorphic. They may appear as small round cells with scanty cytoplasm and dense chromatin, resembling lymphoblasts, or as larger cells with a fine chromatin pattern and prominent nucleoli (Figs. 6.13.2 and 6.13.3) (1,7,11). The large-cell type usually has a moderate amount of basophilic cytoplasm with or without azurophilic granules. The most specific morphologic feature is the presence of cytoplasmic blebs (budding), which represents the process of platelet shedding from the cell surface. However, the real process is seen only in mature megakaryocytes. Cytoplasmic blebs, nevertheless, can be an artifact seen in other subtypes of AML and is thus not pathognomonic for AMKL.

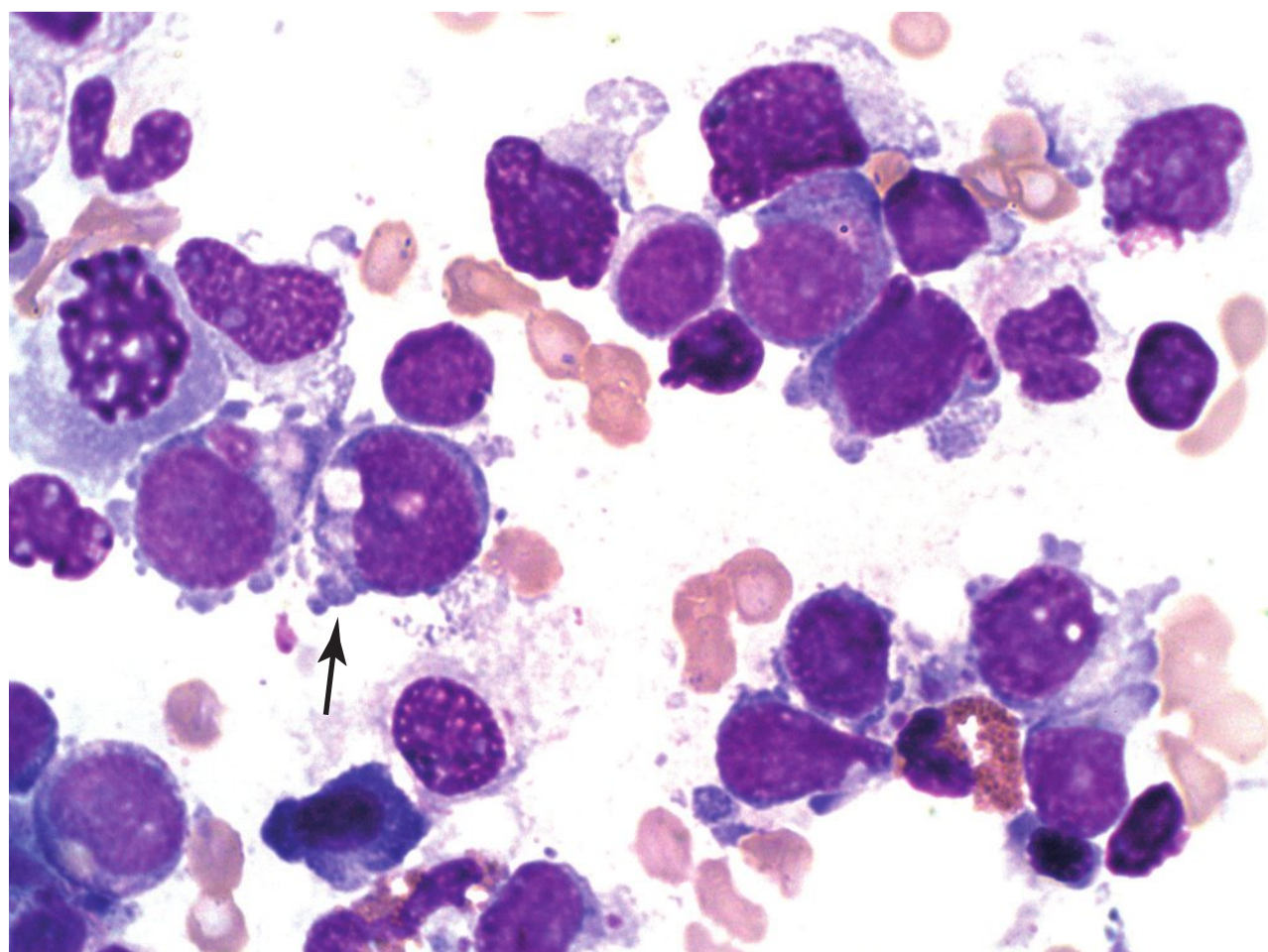


FIGURE 6.13.2 Bone marrow aspirate shows a cluster of megakaryoblasts of varying sizes. Large cells show moderate amount of basophilic cytoplasm with irregular cell border or budding (arrow). Chromatin is dispersed and nucleoli are present. Small cells, in contrast, reveal scanty cytoplasm and dense chromatin, resembling lymphoblasts. Wright-Giemsa, 100× magnification.

Megakaryoblasts may be difficult to identify in bone marrow biopsy, but their presence is usually suggested by the accompanying megakaryocytes and reticular fibrosis (Figs. 6.13.4 and 6.13.5). The megakaryocytes are frequently smaller than normal megakaryocytes, about 7 to 10 mm in diameter, hypolobated, or mononucleated (12). These megakaryocytes are often referred to as micromegakaryocytes or dwarf megakaryocytes. They may be present singly or forming small or large clusters. A reticulin fiber network usually surrounds the mature megakaryocytes, but prominent fibrosis may not be present, especially in cases where immature megakaryoblasts are predominant.

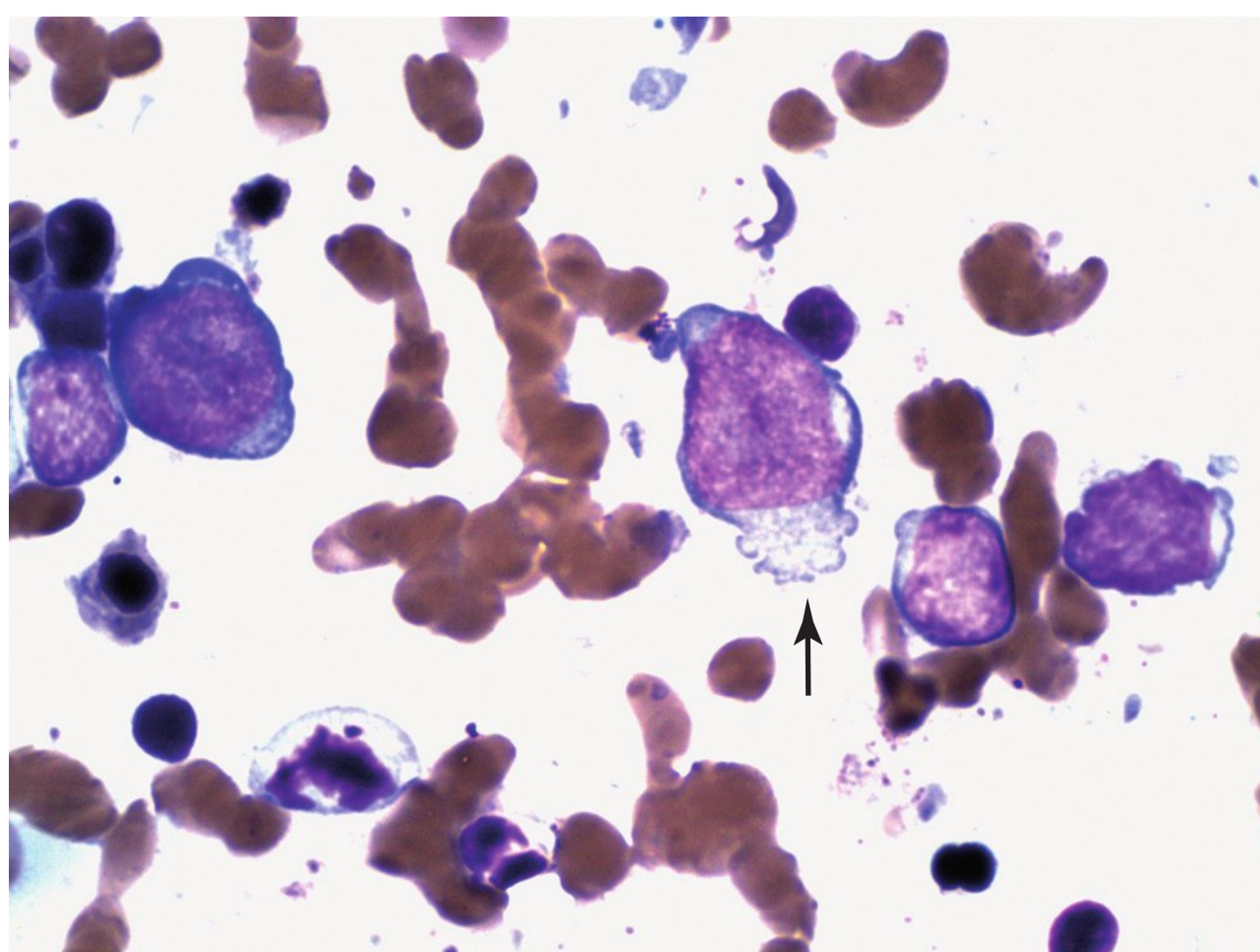


FIGURE 6.13.3 Bone marrow touch imprint shows two large and two small megakaryoblasts. Cytoplasmic projection is clearly visible in the large blasts (arrow). Wright-Giemsa, 100× magnification.

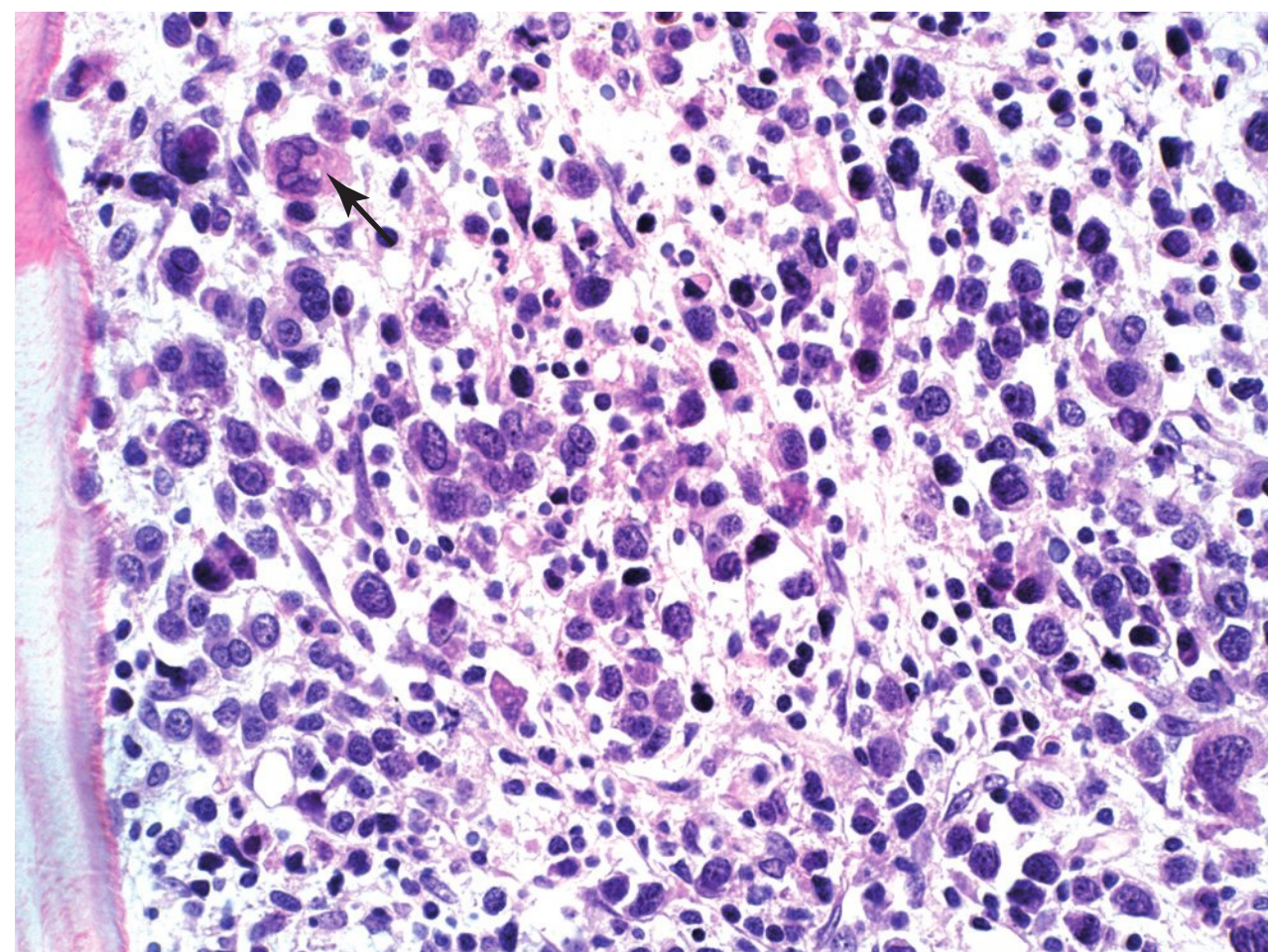


FIGURE 6.13.4 Bone marrow biopsy shows total replacement of the normal hematopoietic cells by megakaryoblasts and a few large megakaryocytes (arrow). Marked variation in size of the blasts distinguishes megakaryoblasts from other blasts. Hematoxylin and eosin, 40× magnification.

In equivocal cases, electron microscopic identification of platelet peroxidase can be helpful. The platelet peroxidase appears earlier than other platelet antigens as identified by other monoclonal or polyclonal antibodies, discussed later (13). In megakaryocytes and megakaryoblasts, the peroxidase reaction is localized on the nuclear membrane and the endoplasmic reticulum (ER), whereas the reaction in myeloblasts mainly occurs in the Golgi area and cytoplasmic granules (Figs. 6.13.6 and 6.13.7) (7).

In cases with a dry tap, the enumeration of the percentage of megakaryoblasts is impossible and the blast count depends on a rough estimation in the core biopsy. Under this condition, many similar diseases should be carefully excluded, such as myelodysplastic syndromes,

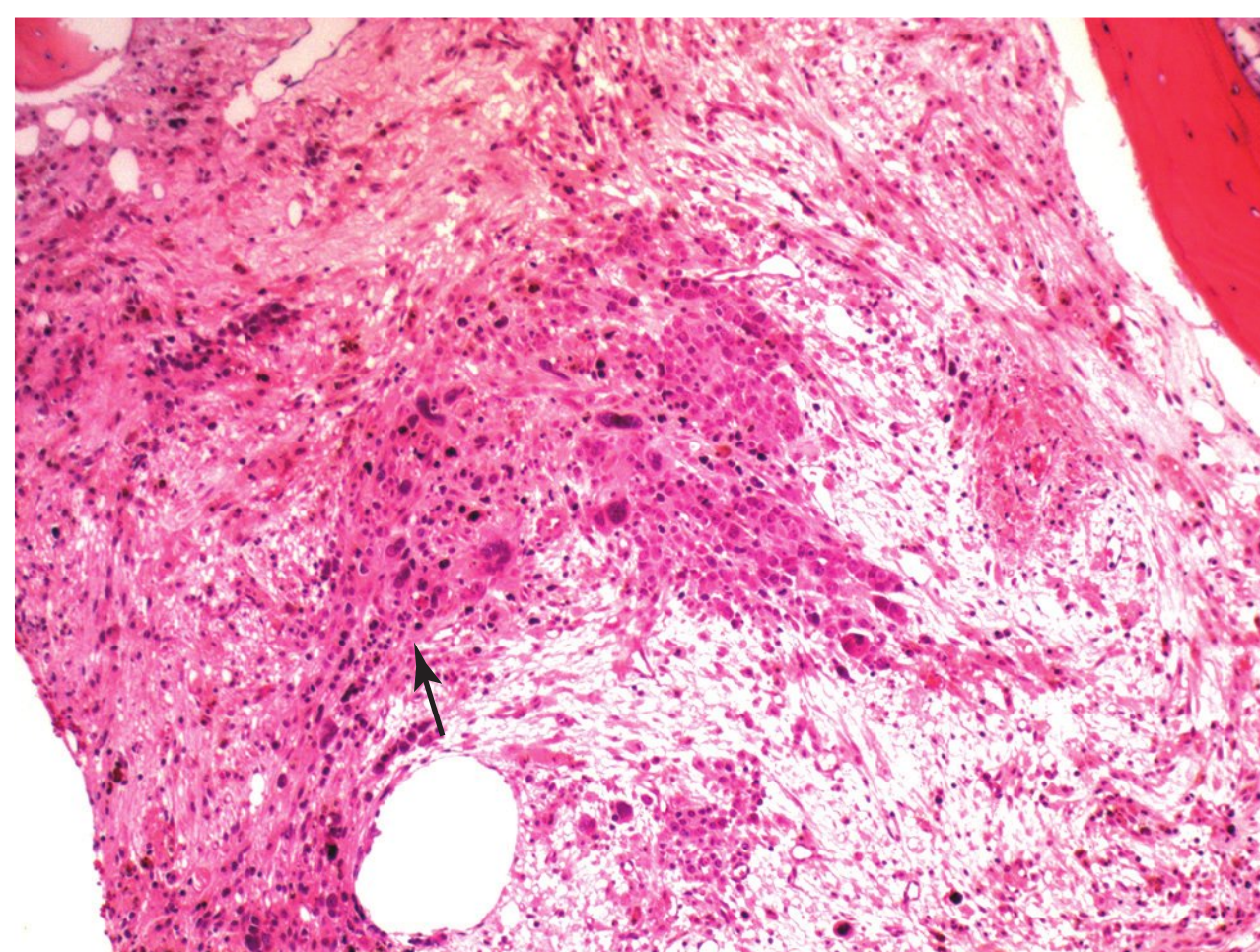


FIGURE 6.13.5 Bone marrow biopsy in a case of M7 with relapse shows a cluster of leukemic cells (arrow) on a fibrotic background. Hematoxylin and eosin, 10× magnification.

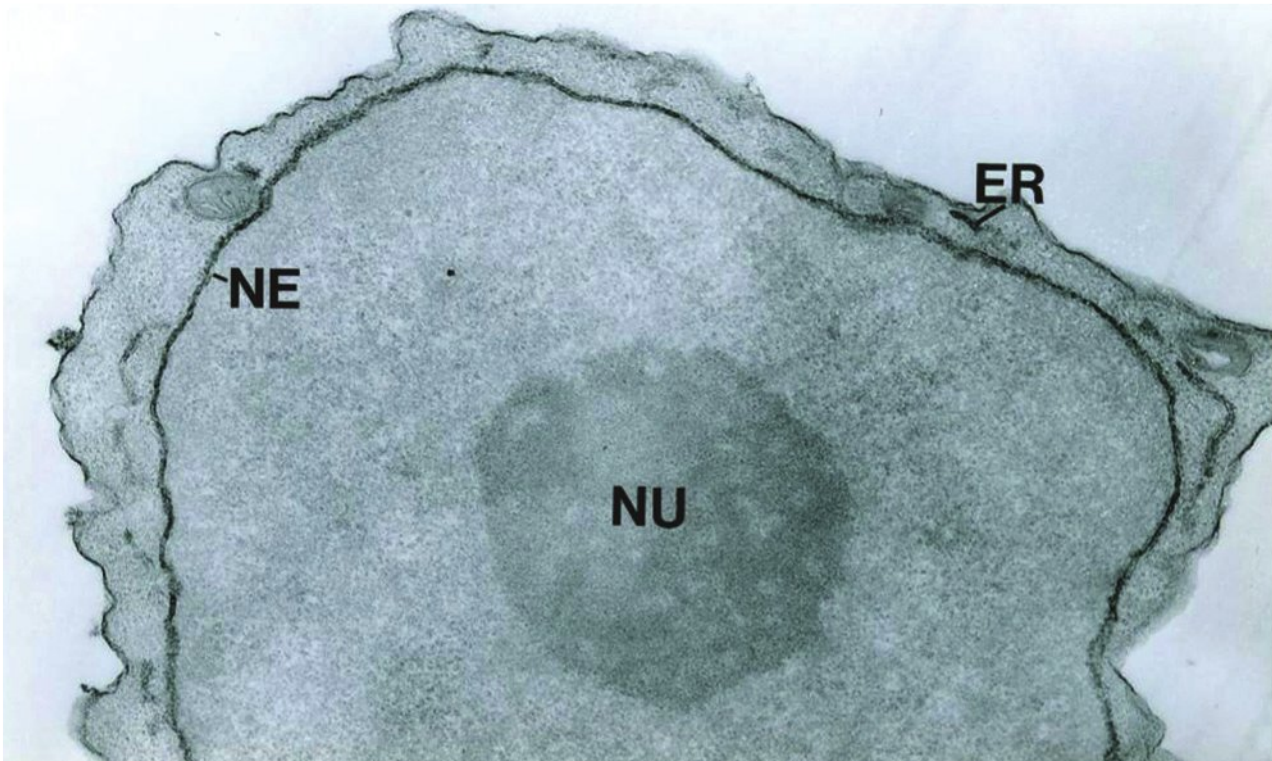


FIGURE 6.13.6 Electron micrograph of a megakaryoblast in a case of M7 leukemia, showing platelet peroxidase activity in the ER including the nuclear envelope (NE). Note the prominent nucleolus (NU) in the nucleus. 30,000× magnification. (Courtesy of Dr. Saul Teichberg, North Shore University Hospital, New York.)

acute panmyelosis with fibrosis, primary myelofibrosis, or other subtypes of AML before or after treatment (14,15). In these diseases, megakaryocytes are a reactive component that may become dysplastic, mimicking malignant cells. The distinction of these entities with AMKL requires multiparameter studies. For instance, a recent comparative study of acute panmyelosis with myelofibrosis and AMKL found that the former is characterized by a multilineage myeloid proliferation, smaller blast population, and infrequent expression of megakaryocytic antigen (16). Making the matter more complicated is the fact that these diseases can transform into each other. A case report of chronic idiopathic myelofibrosis transformed into AMKL is a good example (17). In contrast, AMKL with t(1;22) in an infant may be mistaken as metastatic neuroblastoma (9). Cuneo et al. (18) used 20% platelet antigen–positive cells as a cutoff point to distinguish AMKL from other disorders containing megakaryocytic elements.

Cytochemical stains are characteristic in the constant absence of MPO and Sudan black B stains but in the presence of PAS (Fig. 6.13.8) and acid phosphatase (19,20). The PAS stain is typical in a peripheral staining pattern with prominent staining of the cytoplasmic blebs (20). The reactions of various nonspecific esterases differ: a-naphthyl butyrate esterase is negative but a-naphthyl acetate esterase and naphthol AS-D acetate esterase are positive, with focal staining in megakaryoblasts.

Immunophenotype

The monoclonal antibodies most commonly used for the identification of megakaryocytes are CD41, CD42, and CD61 (1–3,8,14,21). Recently, CD36 has been included in the megakaryocytic profile (4,9). In paraffin sections, factor VIII and von Willebrand factor antibodies are frequently used to identify megakaryocytes, but these antigens may not be present on the leukemic cells in all patients with AMKL (10,13). Currently, CD42b and CD61 antibodies are available for immunohistochemical staining and provide a more specific identification (9).

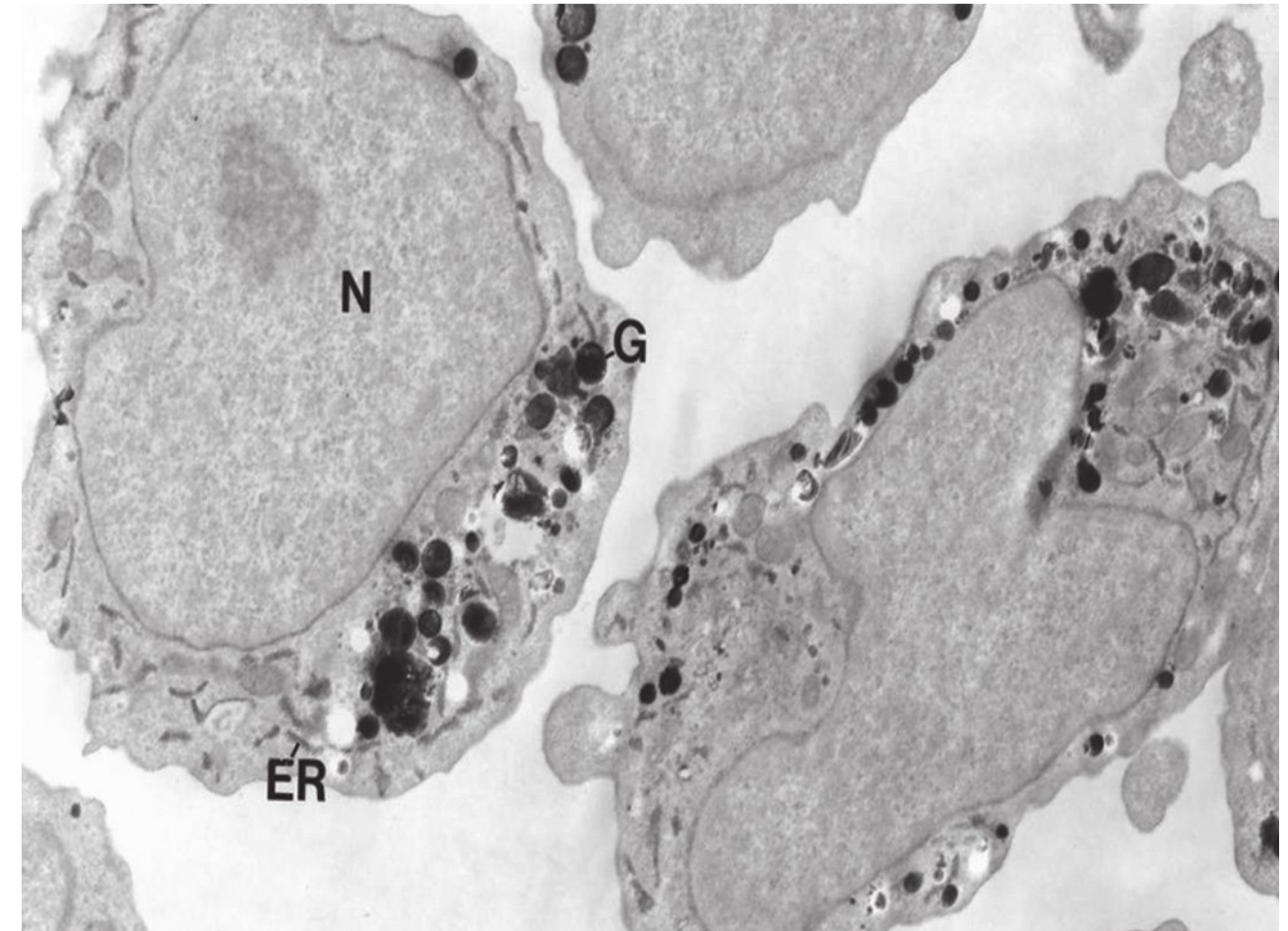


FIGURE 6.13.7 Electron micrograph of a myeloblast showing MPO activity in many secretory granules (G) but also in the ER including the NE. Note the immature nucleus (N) with a prominent nucleolus in one cell. 11,500× magnification. (Courtesy of Dr. Saul Teichberg, North Shore University Hospital, New York.)

A note of caution: Platelets frequently adhere to myeloblasts and cause falsely high percentages of CD41, CD42, and CD61 (22). Therefore, the study of cytoplasmic platelet antigens may avoid this problem (9,10). Some studies mentioned that CD42 is positive in mature platelets but negative in some cases of immature megakaryoblasts (14,23,24); thus, the presence of a low percentage of CD42 as compared to CD41 and CD61 may be a clue to the diagnosis of AMKL. In addition, megakaryocytic reaction is occasionally seen in other subtypes of acute leukemias (Fig. 6.13.9), leading to the demonstration of high percentages of CD41/CD42/CD61-positive populations by flow cytometry.

For myelomonocytic antigens, CD33 is frequently positive, but MPO, CD13, CD14, and CD15 are usually negative (2,18,20,25,26). CD56, a neural cell adhesion molecule and natural killer–cell marker, is frequently expressed on megakaryoblasts, as reported in the earlier but not the current

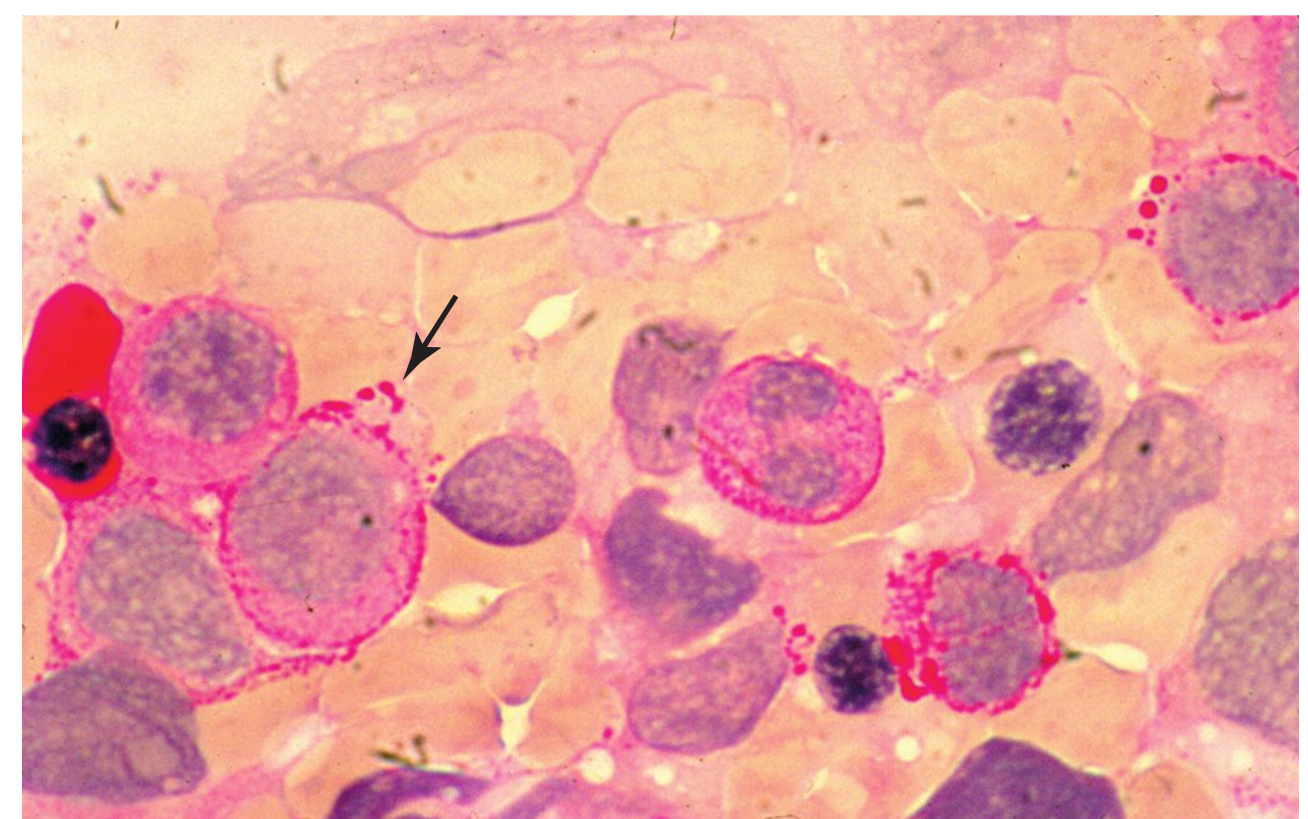


FIGURE 6.13.8 Bone marrow aspirate with PAS stain shows a peripheral staining pattern with accentuation in cytoplasmic blebs (arrow). 100× magnification.

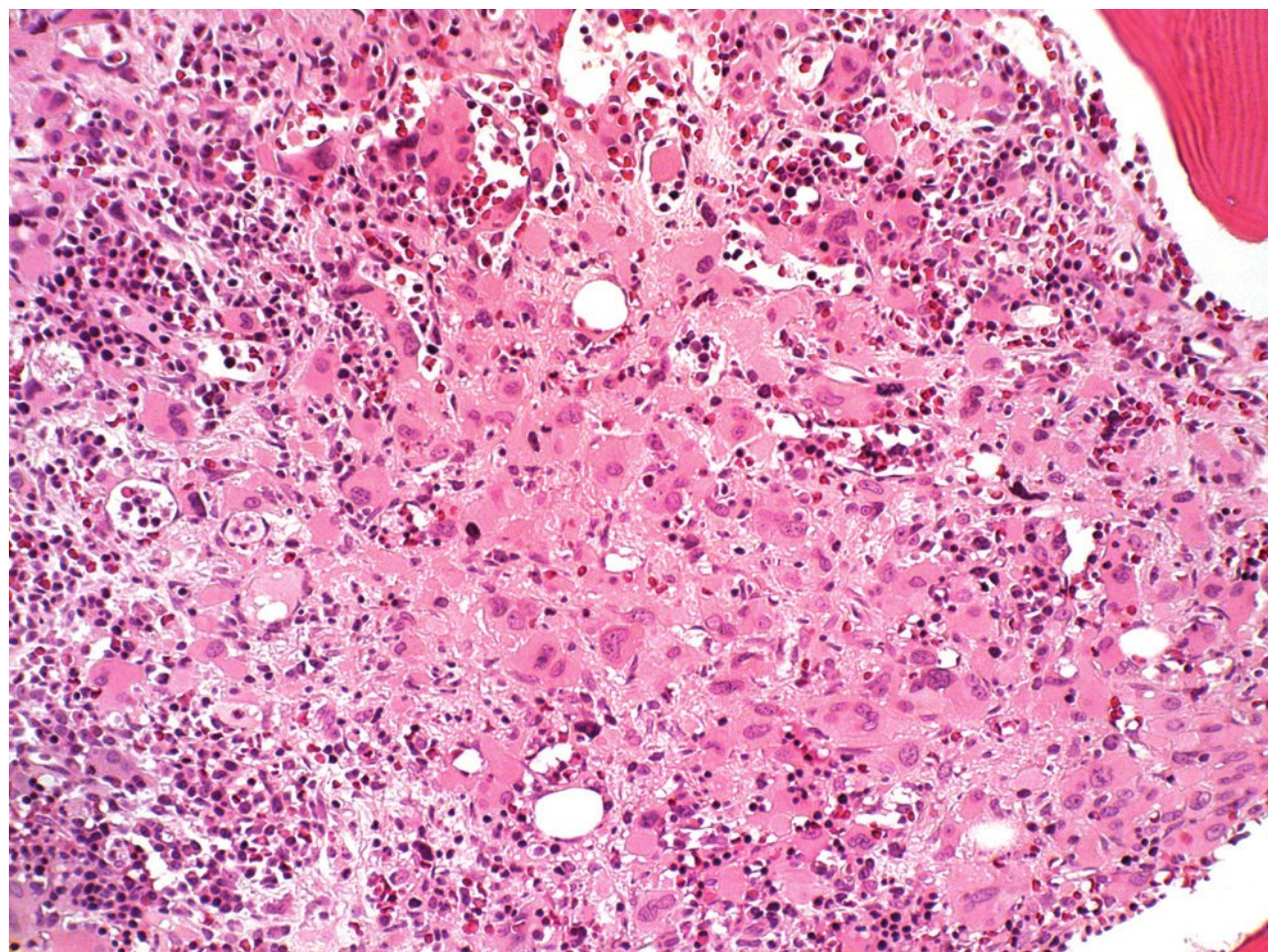


FIGURE 6.13.9 Bone marrow biopsy from a patient with acute myeloblastic leukemia shows extensive megakaryocytic reaction. Hematoxylin and eosin, 20× magnification.

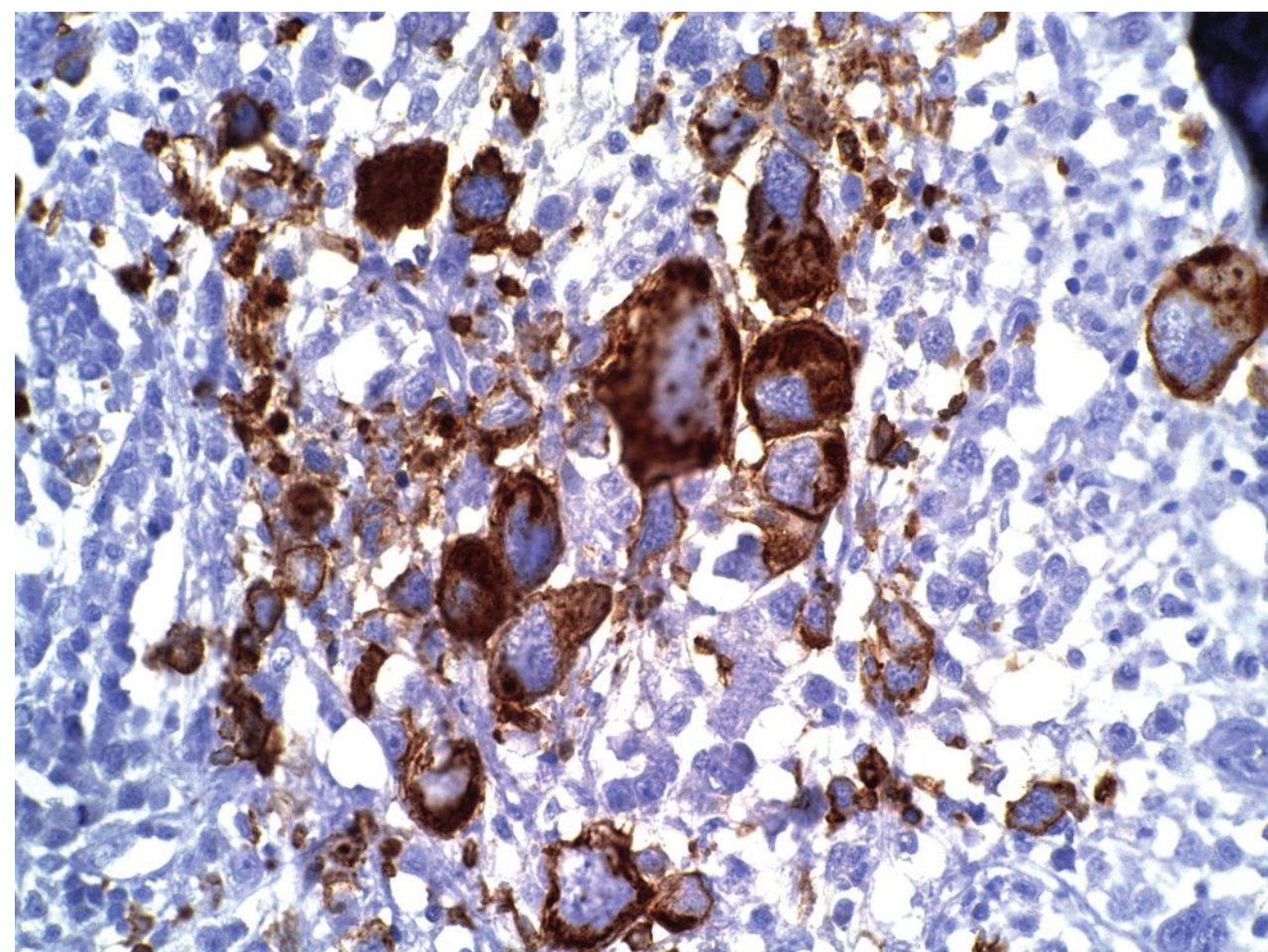


FIGURE 6.13.10 Bone marrow biopsy with CD42b staining shows positive stain in the relatively mature megakaryocytes/megakaryoblasts. MPO, 40× magnification.

literature (10,13,18,20,26–28). In AMKL, not otherwise specified (NOS), CD34 is often negative (10), but in AMKL with DS, CD34 is present in 50% of cases and CD117 is consistently positive (29). The expression of HLA-DR is variable (2,19,20). Glycophorin A has been detected in 18% of 41 cases of pediatric AMKL at St. Jude Children's Research Hospital (4).

For lymphoid markers, the B-cell markers (CD19, CD79a, CD10, and CD20) are generally negative (2,18,20,25). The expression of T-cell markers is selective: CD2 and CD7 are often positive, but CD3 and CD5 are usually negative (4,9,19).

In the current case, the diagnosis was based on the demonstration of typical morphology in some of the megakaryoblasts in the bone marrow aspirate. This diagnosis was further confirmed by the flow cytometric study that demonstrated positive reactions in all three megakaryocytic antigens with a much lower percentage of CD42 than of CD41 and CD61. The bone marrow biopsy showed total replacement of normal hematopoietic cells by the small mononucleated megakaryoblasts with scattered megakaryocytes in various degrees of maturation. Myelofibrosis appeared only after chemotherapy. Immunohistochemical stains in bone marrow core biopsy demonstrated CD42b on the relatively mature megakaryocytes, but not on megakaryoblasts. However, when the patient was in remission, CD42b was not demonstrated in the fibrotic bone marrow. The PAS stain also played a role in the early diagnosis of this case by demonstrating the surface staining pattern on the megakaryoblasts.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is able to identify three megakaryocytic antigens, CD41, CD42, and CD61. The discrepancy between CD42 and the other two markers is helpful in establishing the diagnosis. In immunohistochemistry, the use of CD42b and CD61 may directly identify the megakaryocytes, but the former only identifies the mature cells and not the blastic cells (Fig. 6.13.10). CD61 staining, in contrast, is affected

by the fixation procedure and decalcification (9). Factor VIII staining may be used to supplement CD42b and CD61 in achieving the diagnosis.

Molecular Genetics

As trilineage myelodysplasia is frequently coexistent with de novo AMKL and many of the chromosome aberrations in AMKL are shared by other myeloid neoplasms, this leukemia appears to be derived from a multipotent stem cell (18). The cytogenetic abnormalities are also shared with myelodysplastic syndrome, and dysgranulopoiesis is seen in one third of the pediatric AMKL cases in one study (6). These findings suggest that AMKL may often be a secondary leukemia. Some studies also suggested that megakaryocytes may share a common progenitor cell with erythrocytes (5,26,30).

Cytogenetic analyses have been conducted in many studies of AMKL (3,18,20,25,31,32), but no cytogenetic profile has emerged as a specific marker. By summarizing 31 cases in the literature in addition to their own 15 cases, Cuneo et al. (18) found that -7/7q- and -5/5q- are the most common abnormalities. A French study found AMKL to be characterized by a higher incidence of abnormalities, a higher complexity of karyotypes, and a different distribution of abnormalities among children and adults (6). This study divided AMKL cases into nine cytogenetic groups: (i) normal karyotypes, (ii) patients with DS, (iii) numeric abnormalities only, (iv) t(1;22)(p13;q13), (v) t(9;22)(q34;q11), (vi) 3q21q26, (vii) -5/del(5q) or -7/del(7q) or both, (viii) i(12)(p10), and (ix) other structural changes. Whereas groups 1, 2, 3, and 4 were exclusively seen in children, groups 5, 6, 7, and 8 were mainly seen in adults (6).

Lu et al. (33) found that pediatric patients with DS and those without differ in the karyotypes. In patients with DS, 10 of 43 cases showed no additional cytogenetic abnormalities besides trisomy 21. Most of the patients without DS showed aberrations of chromosome 22, including 16 cases with t(1;22)(q13;q13) and 6 cases with 22q13 translocation

variants. The remaining non-DS patients showed frequent cytogenetic changes, including rearrangement of 3q21, 3q26-27, trisomy 21, and other specific changes.

The karyotype aberration t(1;22) has been exclusively reported in pediatric cases; most patients were younger than 12 months (3,20,32,34). In the 2008 WHO classification, AML with this karyotype has been designated a distinct entity and separated from AMKL, NOS (35). This translocation is estimated to be present in 30% of pediatric patients and in >65% of infants with AMKL (36). This translocation is of special interest because it involves two oncogenes and because of its association with myelofibrosis. Previously, it was considered that the N-ras oncogene, located in the breakpoint 1p13, might be responsible for malignant transformation of megakaryocytes, while c-sis oncogene on chromosome 22q13 that encodes platelet-derived growth factor B, responsible for myelofibrosis (20,37,38). Recently, however, RNA-binding motif protein 15 (RBM15 or OTT) and megakaryocyte leukemia 1 (MKL1 or MAL) genes have been found in 1p13 and 22q13, respectively (39,40). The RBM15-MKL1 fusion transcript can be identified with molecular biology techniques in karyotypically cryptic cases (6). Although the exact role of the RBM15-MKL1 in the pathogenesis of AMKL is still unknown, the fusion gene may modulate chromatin organization, HOX-induced differentiation, and extracellular signaling pathways (39,40). Patients with t(1;22) have a worse prognosis than do those AMKL cases without this translocation because of their poor response to chemotherapy. Therefore, autologous bone marrow transplantation is the therapy of choice, particularly for this group of patients (3).

AMKL with DS has become a separate entity in the 2008 WHO scheme (29). In this entity, mutation in the gene for globin transcription factor 1 (GATA-1) has been implicated as a major mechanism for leukemogenesis (41). GATA-1 is a transcription factor that is essential for normal megakaryocytopoiesis. Absence of GATA-1 promotes accumulation of immature megakaryocytes. Acquired mutations in GATA-1 have been detected in the vast majority of AMKL patients with DS and in almost all cases of transient myeloproliferative disorder (41). However, trisomy 21 and GATA-1 mutations together are still insufficient to cause AMKL in DS patients, and additional genetic or epigenetic events are required for final malignant transformation (42,43). The recent identification of activating mutations in several candidate genes, including KIT, FLT3, JAK2, JAK3, and MPL, may play a role in leukemogenesis of AMKL, but further studies are required for confirmation (42–45). Another new discovery is that a microRNA, miR125b-2, is a positive regulator of megakaryopoiesis and an oncomiR involved in the pathogenesis of trisomy-associated AMKL (46).

The salient features for laboratory diagnosis of AMKL are summarized in Table 6.13.1.

Clinical Manifestations

As mentioned before, AMKL can be seen in different age groups that may have biologic, cytogenetic, and prognostic differences. In adults, AMKL is frequently secondary to

TABLE 6.13.1

Salient Features for Laboratory Diagnosis of AMKL

1. Presence of $\geq 20\%$ megakaryoblasts in bone marrow aspirate
2. If bone marrow aspirate is not successful, bone marrow biopsy identification of immature leukemic cell infiltration and immunologic identification of megakaryocytes in blood or bone marrow are required.
3. Flow cytometry shows high percentages of CD41 and CD61 but low percentage of CD42b.
4. Immunohistochemical stains including CD42b, CD61, and factor VIII are available.
5. Electron microscopic identification of platelet peroxidase in leukemic cells
6. A peripheral PAS staining pattern or staining of the blebs on the blasts
7. t(1;22)(p13;q13) is specific for infantile megakaryoblastic leukemia.
8. Trisomy 21 and GATA-1 mutations are characteristic for AMKL with DS.

chemotherapy, to leukemic transformation of either myelofibrosis or myelodysplastic syndrome, or as megakaryoblastic crisis of chronic myelogenous leukemia (3,7,27). In contrast, AMKL in children generally appears de novo (2).

Children with DS have a 10- to 20-fold increased risk for the development of acute leukemia (24), especially AMKL (18,47,48). In this group of patients, AMKL accounts for approximately 50% of acute leukemia and is frequently preceded by transient leukemia 1 to 4 years earlier (5). In a study of 20 children with DS in Japan, 14 had AMKL and all were younger than 3 years (21). Therefore, the possibility of an association between trisomy 21 and AMKL has been raised. However, trisomy 21 does not frequently appear in AMKL leukemic cells when the patient has a normal constitutional karyotype (21). In contrast, patients with transient leukemia and those with subsequent development to AMKL all have trisomy 21 in their leukemic cells (28). In those patients, if they do not have DS, they may have trisomy 21 mosaicism with a normal karyotype. Because the same cytogenetic abnormalities in addition to trisomy 21 in the leukemic cells of transient leukemia are also found in the leukemic cells of subsequent AMKL, it is suggested that AMKL in these patients arises from transient leukemic cells (28).

Clinically, the patient may have fatigue, weakness, fever, bleeding, ecchymosis, and petechiae, but lymphadenopathy and hepatosplenomegaly are seldom seen (1,49). However, pediatric patients with t(1;22) may often have organomegaly (36). Patients with AMKL are usually anemic and thrombocytopenic. The leukocyte count may be low at the beginning of the disease, but an abrupt and

rapid increase in the number of peripheral blasts is frequently seen in the terminal stage. The platelet aggregation responses may be impaired, and serum lactate dehydrogenase levels are frequently elevated. Bilateral symmetrical periostitis and osteolytic lesions have been observed in children (50).

Some clinical symptoms are defined by cytogenetic aberrations. For instance, patients with t(1;22) translocation have very early onset of AMKL with organomegaly but have no history of transient leukemia and myelodysplastic syndrome (34). Patients with the 3q21 aberration often have a secondary leukemia with weakness, anemia, CD34+ blasts, marked dysmorphic megakaryoblasts, normal or increased platelet counts, and very poor response to chemotherapy (51,52). However, patients with t(1;22) and 3q21 aberrations are classified as distinct entities in the 2008 WHO scheme (35).

AMKL may be coexistent with other leukemias. A spontaneous and simultaneous occurrence of multiple myeloma and AMKL in a case of polycythemia vera has been reported (53). Another case report described coexistence of acute megakaryoblastic and B-lymphoblastic mixed blast crisis of chronic myeloid leukemia with chronic lymphocytic leukemia (54).

AMKL cases usually have a rapidly progressive course. All patients without treatment die within 1 year. At St. Jude Children's Research Hospital, the 2-year event-free survival in patients with DS is 83%, whereas it is 14% for pediatric cases with de novo AMKL and 20% for cases with secondary AMKL (4). Allogeneic transplantation during remission offers the best chance of cure in this hospital. However, AMKL with DS and AMKL with t(1;22), especially the former, fare much better than AMKL NOS.

REFERENCES

1. Matsuo T, Bennett JM. Nonlymphocytic leukemias and myelodysplastic syndromes. Acute leukemia of megakaryocyte lineage (M7). *Cancer Genet Cytogenet*. 1988;34:1–3.
2. San Miguel JF, Gonzalez M, Canizo MC, et al. Leukemia with megakaryoblastic involvement. Clinical, hematologic and immunologic characteristics. *Blood*. 1988;72:402–407.
3. Lion T, Haas OA, Harbott J, et al. The translocation t(1;22)(p13;q13) is a non-random marker specifically associated with acute megakaryocytic leukemia in young children. *Blood*. 1992;79:3325–3330.
4. Athale UH, Razzouk BI, Raimondi SC, et al. Biology and outcome of childhood acute megakaryoblastic leukemia: a single institution's experience. *Blood*. 2001;97:3727–3732.
5. Zipursky A, Poon A, Doyle J. Leukemia in Down's syndrome. A review. *Pediatr Hematol Oncol*. 1992;9:139–149.
6. Dustugue N, Lafage-Pochitaloff M, Pages MP, et al. Cytogenetic profile of childhood and adult megakaryoblastic leukemia (M7): a study of the Groupe Francais de Cytogenetique Hematologique (GFCH). *Blood*. 2002;100:618–626.
7. Bennett JM, Catovsky D, Daniel MT, et al. Criteria for the diagnosis of acute leukemia of megakaryocyte lineage (M7). A report of the French-American-British Cooperative Group. *Ann Intern Med*. 1985;103:460–462.
8. Brunning RD. Acute myeloid leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1667–1715.
9. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia not otherwise categorized. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:91–105.
10. Arber DA, Brunning RD, Orazi A, et al. Acute megakaryoblastic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:136–137.
11. Gassmann W, Löffler H. Acute megakaryoblastic leukemia. *Leuk Lymphoma*. 1995;18(suppl 1):69–73.
12. Brunning RD, McKenna RW. *Tumours of the Bone Marrow*. Armed Forces Institute of Pathology (AFIP) Fascicle 9, 3rd series. Washington, DC: AFIP; 1994:77–85.
13. Koike T, Aoki S, Maruyama S, et al. Cell surface phenotyping of megakaryoblasts. *Blood*. 1987;69:957–960.
14. Bloomfield CD, Brunning RD. FAB M7. Acute megakaryoblastic leukemia—beyond morphology. *Ann Intern Med*. 1985;103:450–452.
15. Rosenthal NS, Farhi DC. Dysmegakaryopoiesis resembling acute megakaryoblastic leukemia in treated acute myeloid leukemia. *Am J Clin Pathol*. 1991;95:556–560.
16. Orazi A, O'Malley DP, Jiang J, et al. Acute panmyelosis with myelofibrosis: an entity distinct from acute megakaryoblastic leukemia. *Mod Pathol*. 2005;18:603–614.
17. Hirose Y, Masaki Y, Shimoyama K, et al. Granulocytic sarcoma of megakaryoblastic differentiation in the lymph nodes terminating as acute megakaryoblastic leukemia in a case of chronic idiopathic myelofibrosis persisting for 16 years. *Eur J Haematol*. 2001;67:194–198.
18. Cuneo A, Mecucci C, Kerin S, et al. Multipotent stem cell involvement in megakaryoblastic leukemia. Cytologic and cytogenetic evidence in 15 patients. *Blood*. 1989;74:1781–1790.
19. Tallman MS, Neuberg D, Bennett JM, et al. Acute megakaryoblastic leukemia. The Eastern Cooperative Oncology Group experience. *Blood*. 2000;96:2405–2411.
20. Washio S, Ido M, Azuma E, et al. Acute megakaryoblastic leukemia with translocation t(1;22)(p13;q13) in a 10-week-old infant. *Am J Hematol*. 1992;39:56–60.
21. Kojima S, Matsuyama T, Sato T, et al. Down's syndrome and acute leukemia in children. An analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction. *Blood*. 1990;76:2348–2353.
22. Betz SA, Foucar K, Head D, et al. False positive flow cytometric platelet glycoprotein IIb/IIIa expression in myeloid leukemias secondary to platelet adherence to blasts. *Blood*. 1992;79:2399–2403.
23. Borowitz MJ, Bray R, Gascoyne R, et al. U.S.-Canadian consensus recommendations in the immunophenotypic analysis of hematologic neoplasia by flow cytometry. Data analysis and interpretation. *Cytometry*. 1997;30:236–244.
24. Fong C, Brodeur GM. Down's syndrome and leukemia. Epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. *Cancer Genet Cytogenet*. 1987;28:55–76.
25. Slav I, Urban C, Haas OA, et al. Acute megakaryocytic leukemia in children. Clinical, immunologic and cytogenetic findings in two patients. *Cancer*. 1991;68:2266–2272.
26. Cripe LD, Hromas R. Malignant disorders of megakaryocytes. *Semin Hematol*. 1998;35:200–209.
27. Ikushima S, Yoshihara T, Matsumura T, et al. Expression of CD56/NCAM in hematopoietic malignant cells. A usual marker for acute monocytic and megakaryocytic leukemias. *Int J Hematol*. 1991;54:395–403.

28. Zipursky A. Transient leukaemia—A benign form of leukaemia in newborn infants with trisomy 21. *Br J Haematol*. 2001;120:930–938.
29. Baumann I, Niemeyer CM, Brunning RD, et al. Myeloid proliferations related to Down syndrome. In Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008;142–144.
30. Ito E, Kasai M, Toki T, et al. Expression of erythroid-specific genes in megakaryoblastic disorders. *Leuk Lymphoma*. 1996;23:545–550.
31. Sait SNJ, Brecher ML, Green DM, et al. Translocation t(1;22) in congenital acute megakaryocytic leukemia. *Cancer Genet Cytogenet*. 1988;34:277–280.
32. Koller U, Haas OA, Ludwig WD, et al. Phenotypic and genotypic heterogeneity in infant acute leukemia. II. Acute non-lymphoblastic leukemia. *Leukemia*. 1989;3:708–714.
33. Lu G, Altman AJ, Benn PA. Review of the cytogenetic changes in acute megakaryoblastic leukemia. One disease or several? *Cancer Genet Cytogenet*. 1993;67:81–89.
34. Bernstein J, Dastugue N, Haas OA, et al. Nineteen cases of the t(1;22)(p13;q13) acute megakaryoblastic leukemia of infants/children and a review of 39 cases: report from a t(1;22) study group. *Leukemia*. 2000;14:216–218.
35. Arber DA, Brunning RD, Le Beau MM, et al. Acute myeloid leukaemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2);RPN1-EVI1. In: Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008;116–117.
36. Lion T, Haas OA. Acute megakaryocytic leukemia with the t(1;22)(p13;q13). *Leuk Lymphoma*. 1993;11:15–20.
37. Sunami S, Fuse A, Simizu B, et al. The c-sis gene expression in cells from a patient with acute megakaryoblastic leukemia with Down's syndrome. *Blood*. 1987;70:368–371.
38. Marcus RE, Hibbin JA, Matute E, et al. Megakaryoblastic transformation of myelofibrosis with expression of c-sis oncogene. *Scand J Haematol*. 1986;36:186–193.
39. Mercher T, Coniat MB, Monni R, et al. Involvement of a human gene related to the Drosophila spen gene in the recurrent t(1;22) translocation of acute megakaryocytic leukemia. *Proc Natl Acad Sci U S A*. 2001;98:5776–5779.
40. Ma Z, Morris SW, Valentine V, et al. Fusion of two novel genes, RBM15 and MKL1, in the t(1;22)(p13;q13) of acute megakaryoblastic leukemia. *Nat Genet*. 2001;28:220–221.
41. Gurbuxani S, Vyas P, Crispino JD. Recent insights into the mechanisms of myeloid leukemogenesis in Down syndrome. *Blood*. 2004;103:399–406.
42. Roy A, Roberts I, Norton A, et al. Acute megakaryoblastic leukaemia (AMKL) and transient myeloproliferative disorder (TMD) in Down syndrome: a multi-step model of myeloid leukaemogenesis. *Br J Haematol*. 2009;147:3–12.
43. Malinge S, Izraeli S, Crispino JD. Insights into the manifestations, outcomes and mechanisms of leukemogenesis in Down syndrome. *Blood*. 2009;113:2619–2628.
44. Malinge S, Ragu C, Della-Valle V, et al. Activating mutations in human acute megakaryoblastic leukemia. *Blood*. 2008;112:4220–4226.
45. Hussein K, Bock O, Theophile K, et al. MPL^{W515L} mutation in acute megakaryoblastic leukaemia. *Leukemia*. 2009;23:852–855.
46. Klusmann JH, Li Z, Bohmer K, et al. miR-125b-2 is a potential oncomiR on human chromosome 21 in megakaryoblastic leukemia. *Genes Dev*. 2010;24:478–490.
47. Hayashi Y, Eguchi M, Suggita K, et al. Cytogenetic findings and clinical features in acute leukemia and transient myeloproliferative disorders in Down's syndrome. *Blood*. 1989;74:1781–1790.
48. Richard G, Brody J, Sun T. A case of acute megakaryocytic leukemia with hematogones. *Leukemia*. 1993;7:1900–1903.
49. Peterson BA, Levine EG. Uncommon subtypes of acute nonlymphocytic leukemia. Clinical features and management of FAB M5, M6 and M7. *Semin Oncol*. 1987;14:425–434.
50. Athale UH, Kaste SC, Razzouk BI, et al. Skeletal manifestations of pediatric acute megakaryoblastic leukemia. *J Pediatr Hematol Oncol*. 2002;24:561–565.
51. Rynditch A, Schnittger S, Gardiner K. Leukemia breakpoint region in 3q21 is gene rich. *Gene*. 1997;193:49–57.
52. Bitter MA, Neilly ME, LeBeau MM, et al. Rearrangement of chromosome 3 involving 3q21 and 3q26 are associated with normal or elevated platelet counts in acute nonlymphocytic leukemia. *Blood*. 1985;66:1362–1370.
53. Terpstra WE, Meuwissen OJAT, Hagemeijer A, et al. Multiple myeloma and acute megakaryoblast leukemia in spent phase polycythemia vera. *Am J Clin Pathol*. 1990;94:786–790.
54. Colla S, Sammarelli G, Crugnola M, et al. Co-existence of Philadelphia chromosome positive acute megakaryoblastic and B-lymphoblastic mixed blast crisis of chronic myeloid leukemia with chronic lymphocytic leukemia. *Eur J Haematol*. 2004;72:361–365.

CASE 14

Myeloid Sarcoma

CASE HISTORY

A 60-year-old man presented with a 3-week history of increasing fatigue and right neck swelling. Examination of peripheral blood revealed pancytopenia. The patient was treated with antibiotics for neutropenic fever without response. He was admitted to the hospital for further evaluation.

On admission, his total leukocyte count was 9,200/mL with 94% myeloblasts and 6% lymphocytes. Granulocytes were not demonstrated in the peripheral blood. The hemoglobin level was 13.9 g/dL, hematocrit 40.3%, and platelet count 57,000/mL. Physical examination showed no hepatosplenomegaly, but right cervical lymphadenopathy was noted. A lymph node biopsy and a bone marrow biopsy were performed after admission.

The patient was started on imatinib (Gleevec), which was followed by a precipitous drop in the leukocyte count from 1,500 to 600/mL. Within the ensuing days, the patient developed a disseminated fungal infection, renal insufficiency, and altered mental status. He subsequently died.

FLOW CYTOMETRY FINDINGS

Peripheral blood: Myeloid markers: CD13-CD33 86%, CD13-CD33/CD7 80%, CD14 0%, myeloperoxidase (MPO) 0%. Activation antigen: HLA-DR 91%. Immature cell markers: CD34 85%, CD117 66%.

Bone marrow: Myeloid markers: CD13-CD33 86%, CD13-CD33/CD7 82%, CD14 0%, MPO 0%. Activation antigen: HLA-DR 91%. Immature cell markers: CD34 85%, CD117 66%.

Lymph node biopsy: Myeloid markers: CD13-CD33: 96%, CD13-CD33/CD7 95%, CD34 80%, CD117 62% (Fig. 6.14.1).

IMMUNOHISTOCHEMICAL STAINS

The tumor cells showed a strongly positive staining for CD45 and CD43, but were negative for CD3, CD20, CD34, CD45RO, MPO, chloroacetate esterase, and lysozyme.

CYTOGENETIC FINDING

Cytogenetic study showed a normal karyotype of 46,XY in the bone marrow.

DISCUSSION

Myeloid sarcoma (MS) is a solid tumor of extramedullary myeloid cells localized in soft tissues and in bones (Fig. 6.14.2). Extramedullary myeloid leukemic infiltration can be seen in acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), usually found at autopsy, but as far as a tumor mass is not formed, it should not be considered as MS (1,2). This entity was first described by Burn in 1811 (3). The name of chloroma was designated by King (3) because this tumor sometimes shows a green color that fades when exposed to air. The presence or absence of the green color depends on the concentration of MPO in the tumor. By using the peroxidase stain, Bruggess proved the myelogenous origin of chloroma. The term granulocytic

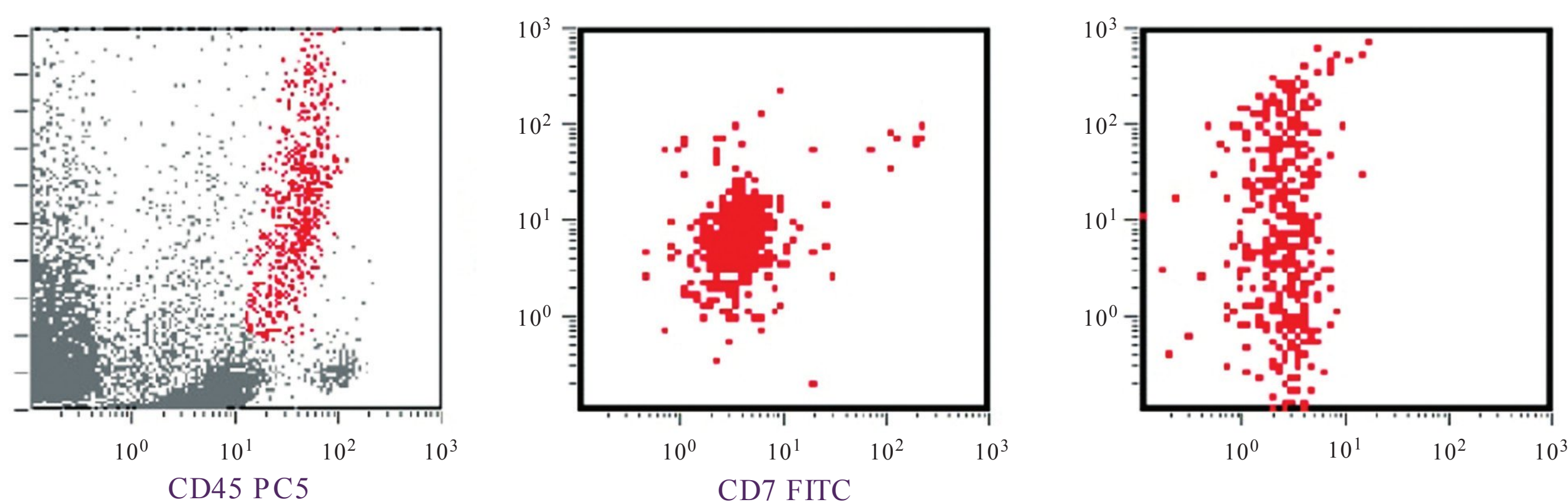


FIGURE 6.14.1 Flow cytometric analysis of a lymph node shows a large cluster of granulocytic cells (red) in the dot plot. Gated cell cluster shows a dual CD7 and CD13-CD33 staining as well as a spectrum of CD34 staining, representing mature and immature granulocytes. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate.



FIGURE 6.14.2 Splenectomy specimen shows a large solid tumor mass in the center, representing an MS.

sarcoma was introduced by Rappaport (3) and had been generally used for many years until recently when the World Health Organization (WHO) scheme designated this tumor as MS (1,4). In the literature, many other synonyms have been used, including extramedullary myeloid cell tumor, myeloblastoma, myelosarcoma, myelomonocytic sarcoma, monocytic sarcoma, and monoblastic sarcoma.

Morphology

MS is morphologically similar to lymphoma, particularly large-cell lymphoma. In three study series, 66%, 75%, and 100% of MS cases, respectively, were initially misdiagnosed as lymphoma (5–7). As will be discussed later, a correct diagnosis requires a high index of suspicion and immunophenotyping of the tumor.

The major clue that may lead to the diagnosis is the demonstration of eosinophilic myelocytes (Fig. 6.14.3) in the hematoxylin and eosin–stained histologic sections (5,6). The presence of the mature granulocytes does not count, because this presence may simply represent a leukocytic reaction in a lymphoma, frequently due to necrosis

of the tumor cells. Unfortunately, about 50% of MS contains no myelocytes. In those cases, a tissue imprint is more helpful for the distinction between lymphoma cells and immature myelomonocytic cells, including myeloblasts, monoblasts, and promyelocytes. Pure monocytic sarcoma was considered rare, but most de novo MSs are of monoblastic origin (8) and most MSs contain a certain percentage of monoblasts (6). The presence of cytoplasmic granules and/or Auer rods is particularly helpful in identifying myeloblasts.

On the basis of tumor-cell differentiation, MS can be classified into three groups: well differentiated, immature (poorly differentiated), and blastic (1,5,9). The well-differentiated group is composed primarily of promyelocytes, but nearly all stages of granulocyte may be present. The immature group consists of myeloblasts and promyelocytes. In tissue sections, the tumor cells have vesicular nuclei with conspicuous nucleoli (Fig. 6.14.4). The nuclei may be variable in size and show nuclear grooves, creases, or convolutions (9). The cytoplasm is moderate to abundant, and a small number of tumor cells may show cytoplasmic granules consistent with myeloid differentiation. The blastic group is formed predominantly of myeloblasts. In tissue sections, the nuclei of the tumor cells are uniform and relatively round. The nuclear chromatin is dispersed, and the nucleoli may or may not be conspicuous. The cytoplasm varies in amount and contains no granules. The monocytic or monoblastic variants may show prominent folded or convoluted nuclei (Fig. 6.14.5).

By immunohistochemical stains, MS can be further divided into different cell lineages, which are designated as granulocytic variant, monoblastic variant, myelomonoblastic variant, megakaryoblastic variant, and erythroblastic variant (10).

MS is usually presented as sheets of leukemic infiltrate, frequently involving adjacent tissues. In the periphery of the tumor mass, tumor cells may form strands and cords (Figs. 6.14.6 and 6.14.7), and sometimes a targetoid pattern,

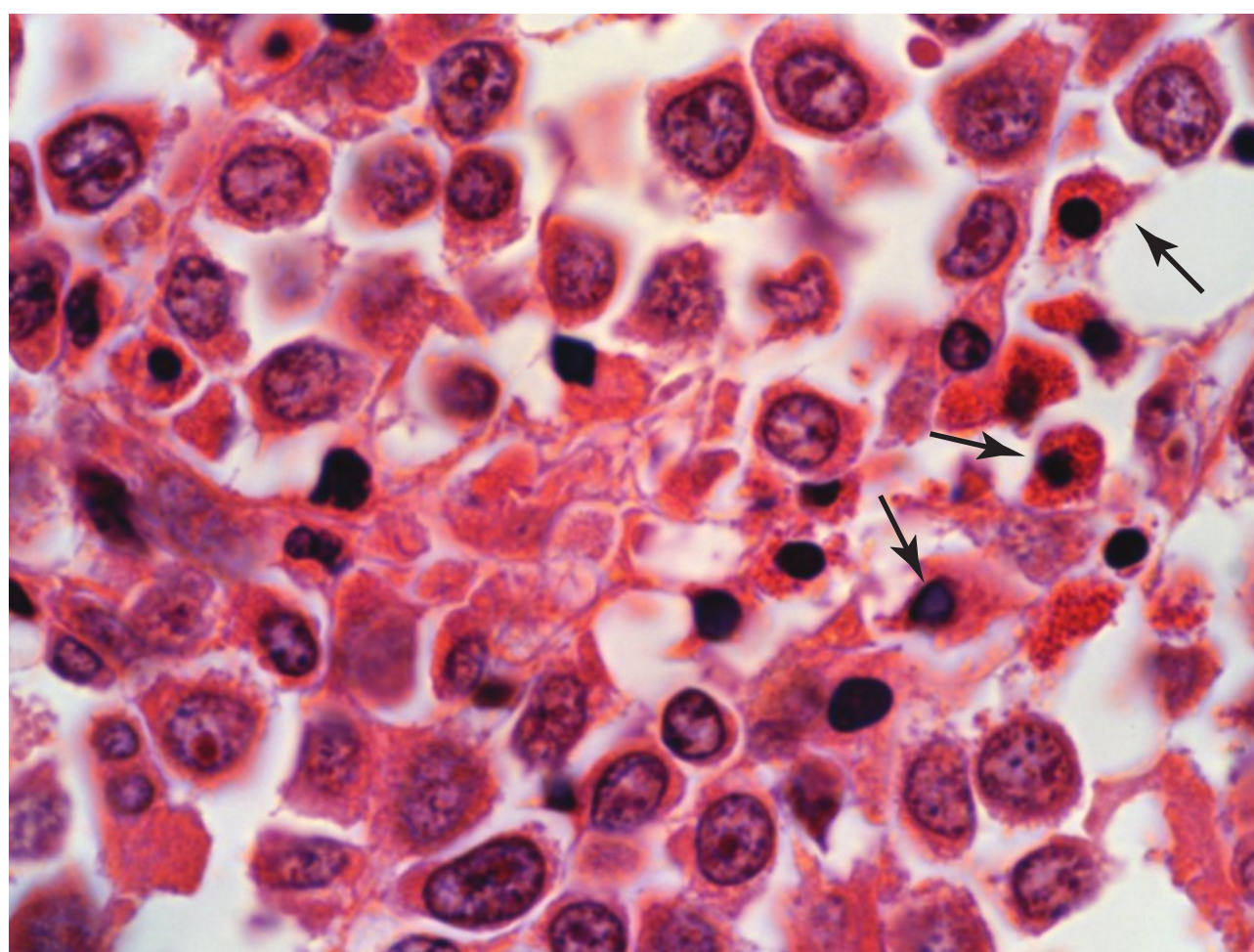


FIGURE 6.14.3 MS of bone shows a few eosinophilic myelocytes (arrows) scattered among the large tumor cells. Hematoxylin and eosin, 100× magnification.

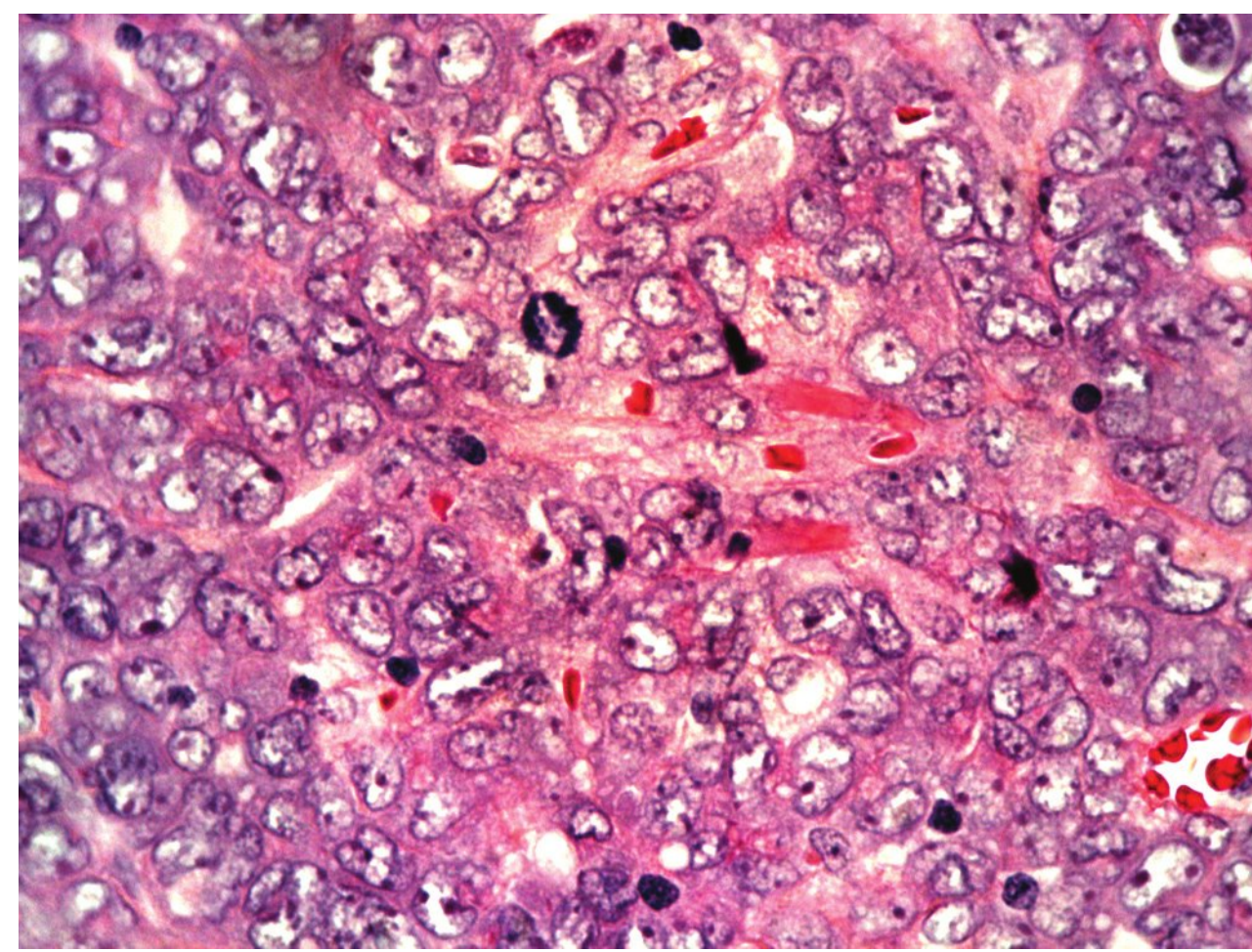


FIGURE 6.14.4 MS of the hip shows large tumor cells with vesicular nuclei and conspicuous nucleoli. Hematoxylin and eosin, 60× magnification.

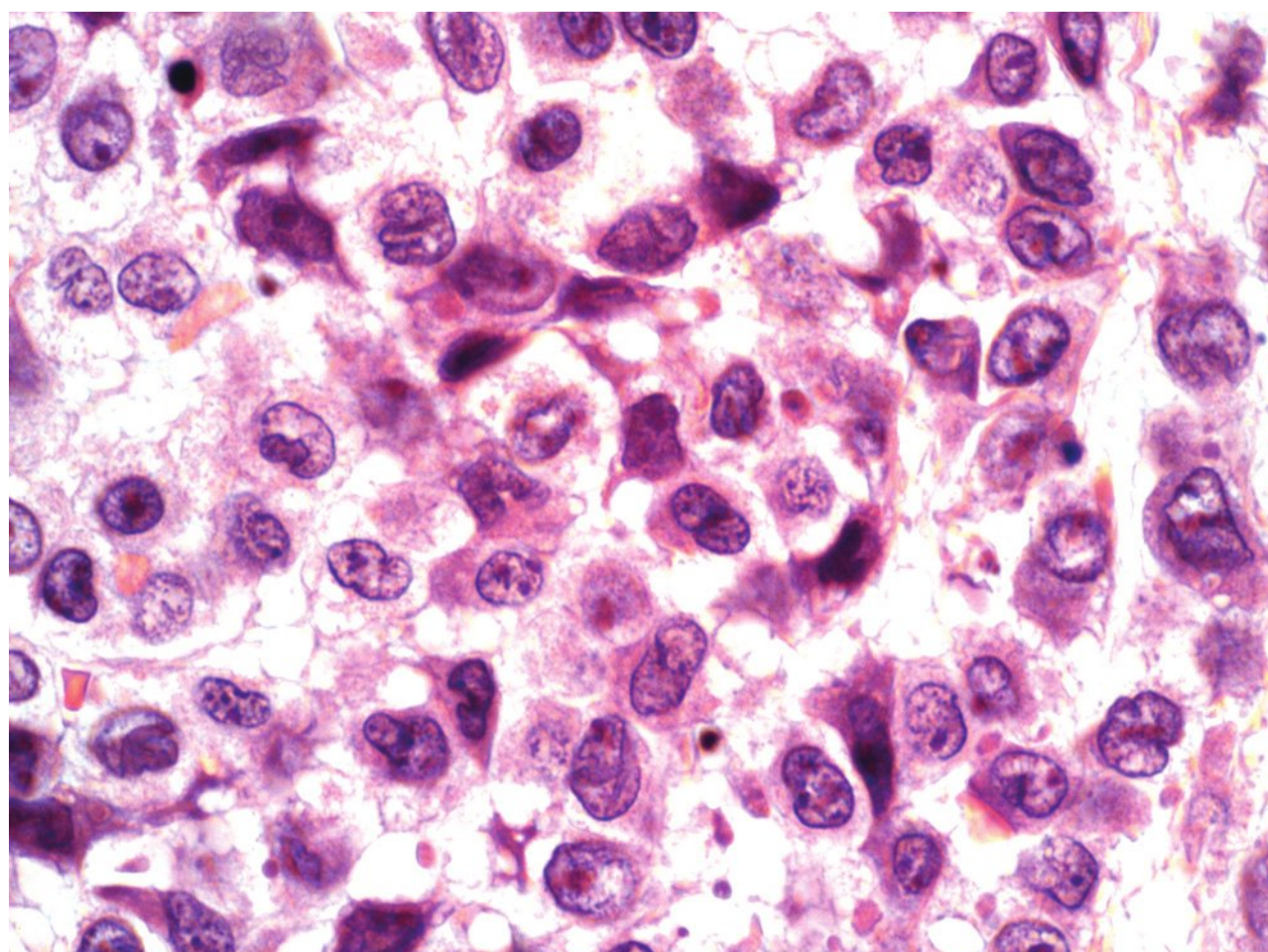


FIGURE 6.14.5 Cutaneous monoblastic sarcoma shows large immature cells with folded or convoluted nuclei. Hematoxylin and eosin, 100× magnification.

vaguely reminiscent of invasive lobular breast carcinoma (6). The tumor infiltrates by expansion, so that normal tissues, such as the glandular and tubular structures, may be separated but the overall architecture is preserved.

In lymph nodes, the sinuses and occasionally the paracortex and medulla are infiltrated by leukemic cells, but the germinal centers are preserved. A reported case of monocytic sarcoma showed a myxoid stroma with cording of tumor cells in the lymph node (11). In another case of MS, the tumor cells assumed plasmacytoid features, simulating nonsecretory multiple myeloma (12). The morphologic characteristics of MS are summarized in Table 6.14.1.

MS may show a starry-sky pattern with a high mitotic rate, mimicking lymphoblastic and Burkitt lymphomas (5,6). Occasionally, MS may also simulate embryonal rhabdomyosarcoma, amelanotic melanoma, or undifferentiated carcinomas (13). Electron microscopy may help in the

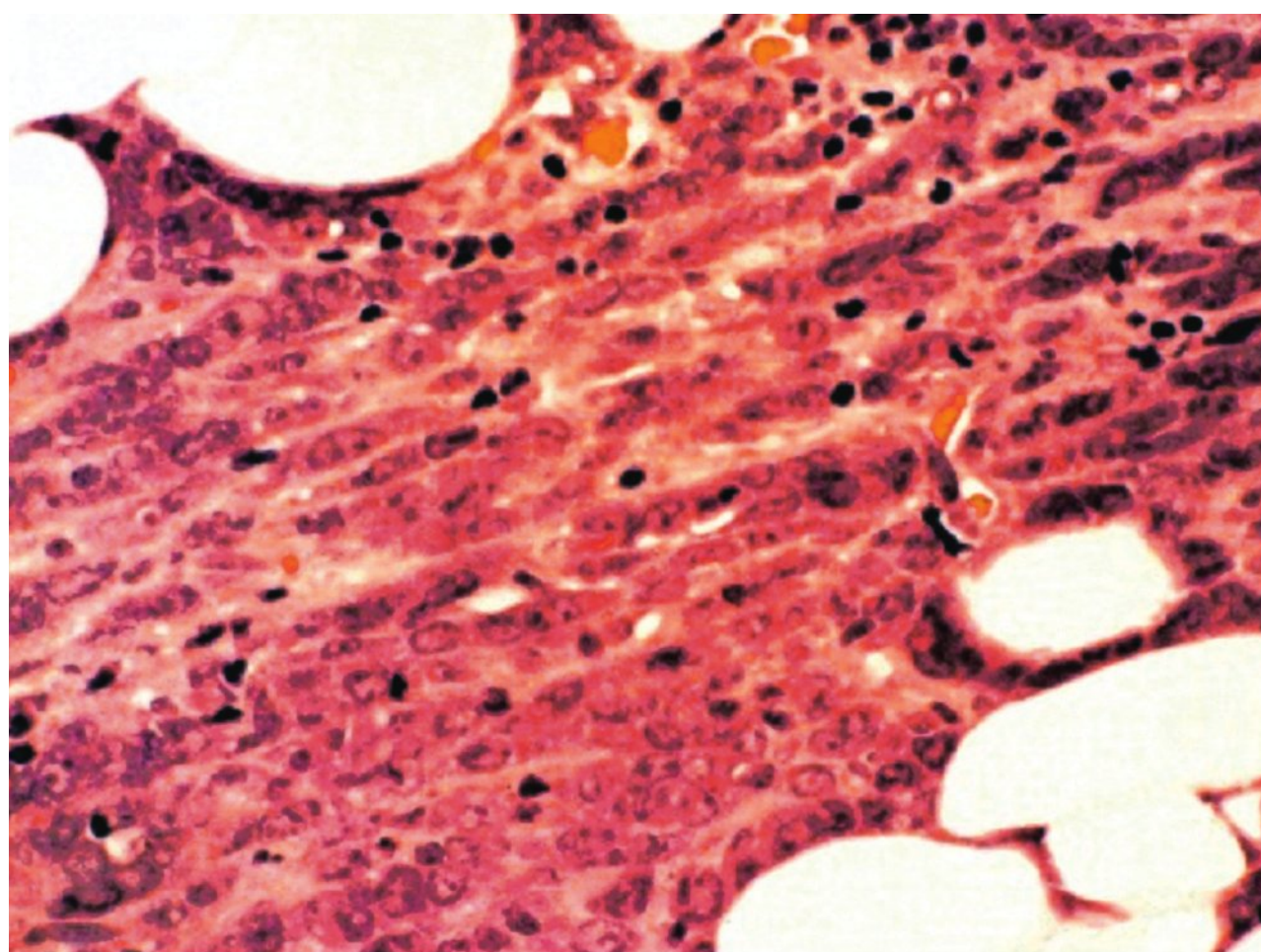


FIGURE 6.14.6 MS of a lymph node shows a cording pattern at the periphery of the tumor. Hematoxylin and eosin, 40× magnification.

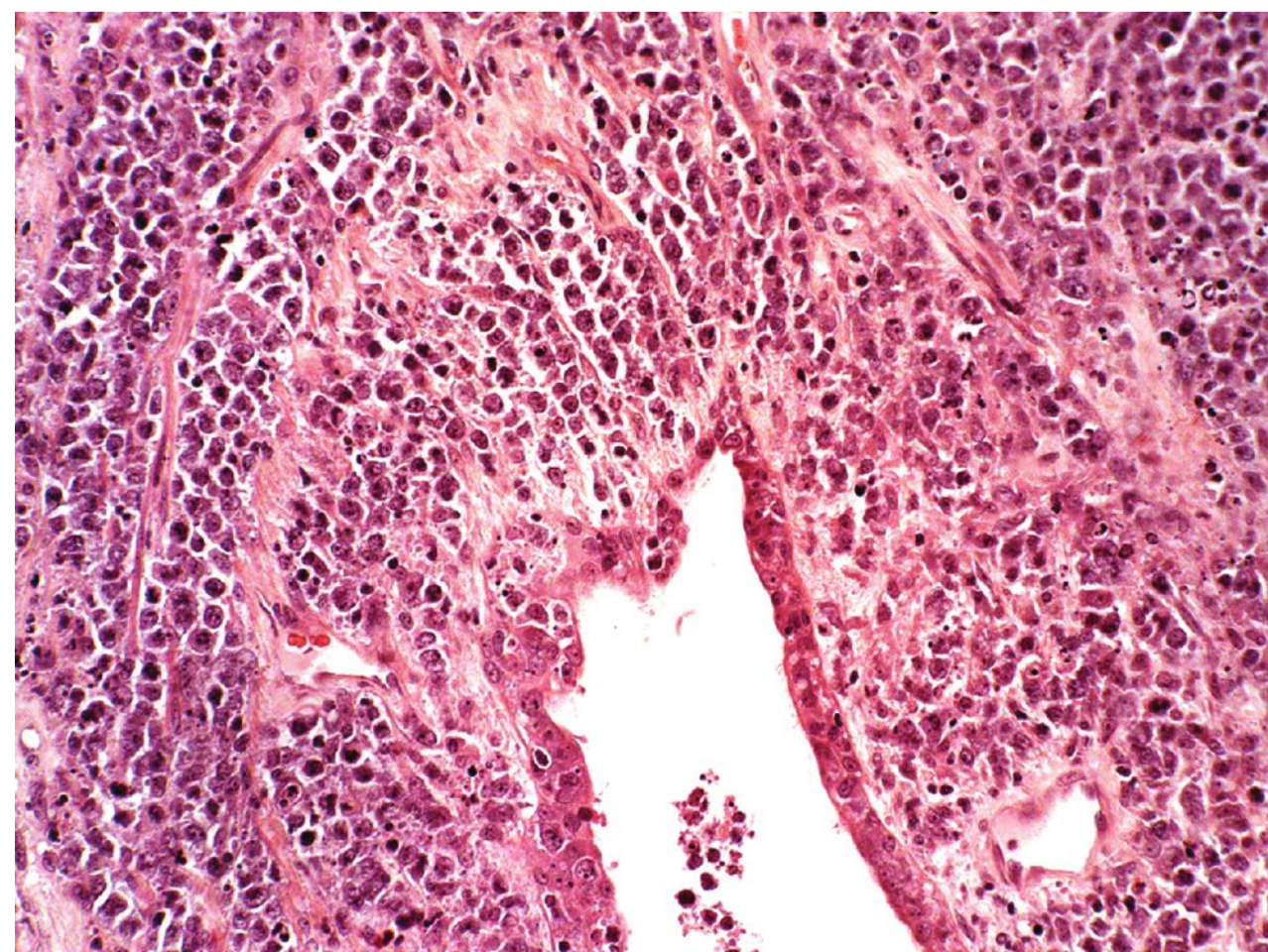


FIGURE 6.14.7 MS of the lung shows cords of tumor cells around the bronchus. Hematoxylin and eosin, 20× magnification.

differential diagnosis by demonstrating specific cytoplasmic granules and/or Auer rods, but the most useful technique for a definitive diagnosis is immunophenotyping by immunohistochemistry or flow cytometry.

Immunophenotype

Most immunophenotypic studies of MS were performed with immunohistochemistry. One of the reasons for this is because MS is usually an unexpected diagnosis, the specimen is often fixed, and by the time MS is suspected, flow cytometry cannot be done. Another reason is that there are many immunohistochemical markers that can be used for the identification of myelomonocytic cells, so that it is more convenient to perform immunohistochemical staining alone.

The time-honored Leder stain for chloroacetate esterase is most frequently used. However, this stain is

TABLE 6.14.1

Characteristic Morphologic Features of MS

Histologic pattern	Sheets of tumor cells separate but do not destroy normal tissue.
Cytology	Tumor cells may form strands and cords at the periphery.
	Tumor cells usually mimic large lymphoma cells, but the scattered eosinophilic myelocytes may give a clue to the diagnosis.
Specific features	Cording arrangement and presence of eosinophilic myelocytes

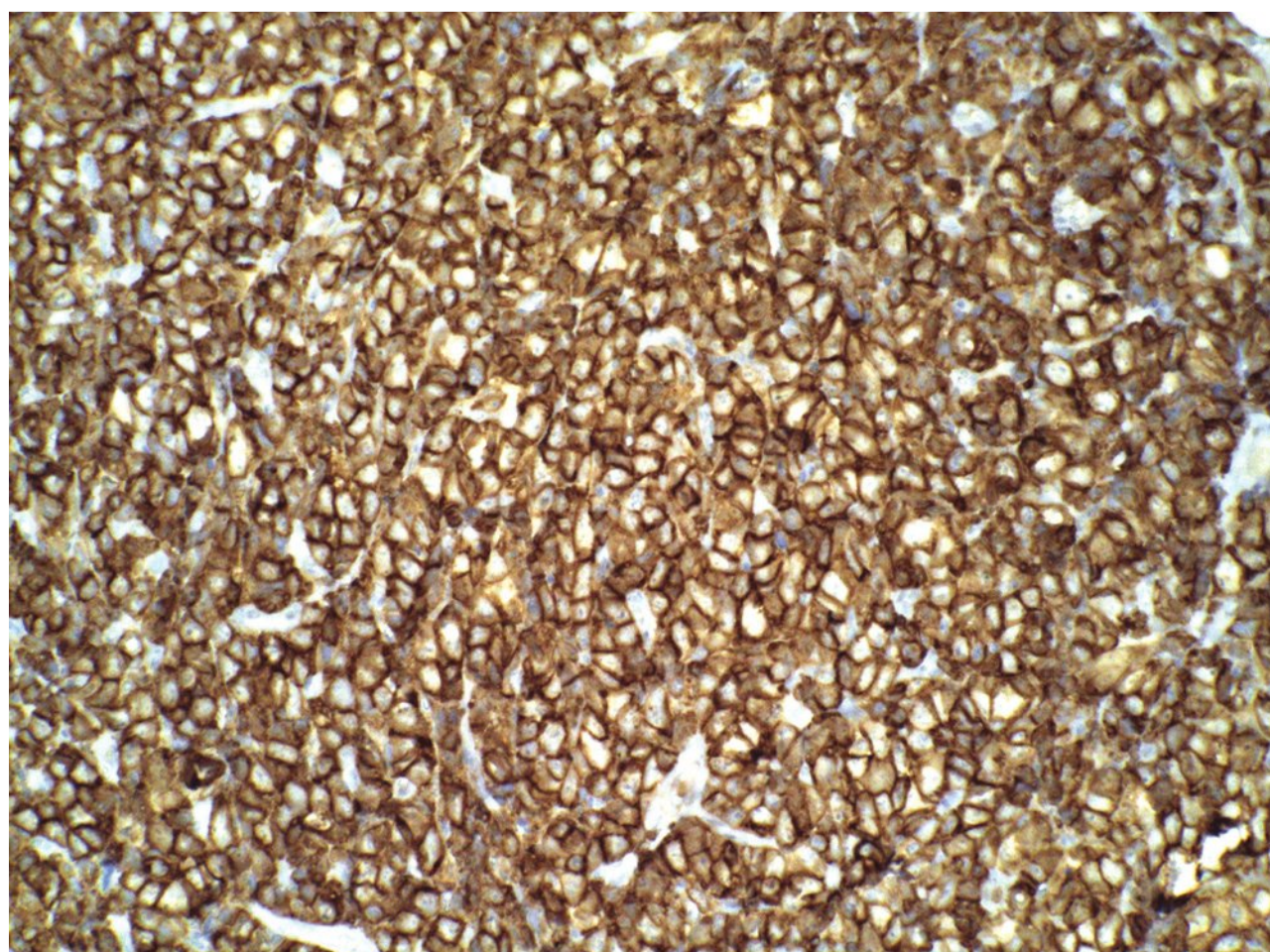


FIGURE 6.14.8 MS cells stain strongly with CD43. Immunoperoxidase, 20× magnification.

relatively insensitive and is frequently negative in tumors composed predominantly of blasts (14). Other common immunohistochemical markers include CD14, CD15, CD43 (Fig. 6.14.8), CD68 (Fig. 6.14.9), MPO, and lysozyme (Fig. 6.14.10). Recently, CD99, CD117, and CD163 have been added to the list (15–17). Additionally, elastase, α_1 -antichymotrypsin, lactoferrin, and cathepsin have been used in rare reports with various sensitivities, but are not accepted as routine histochemical stains (5,9,13,18–20). Audouin et al. (10) have included megakaryoblastic variant of MS and glycophorin C and blood group antigens for the erythroblastic variant.

The sensitivity of some markers depends on the cell type of the tumor. In the study by Traweek et al. (9), CD15 and CD68 (KP-1) were positive for all well-differentiated MS and 76% of poorly differentiated MS. However, for the blastic groups, CD68 was positive in only three of five cases, and CD15 was negative in all of five cases. The insensitivity

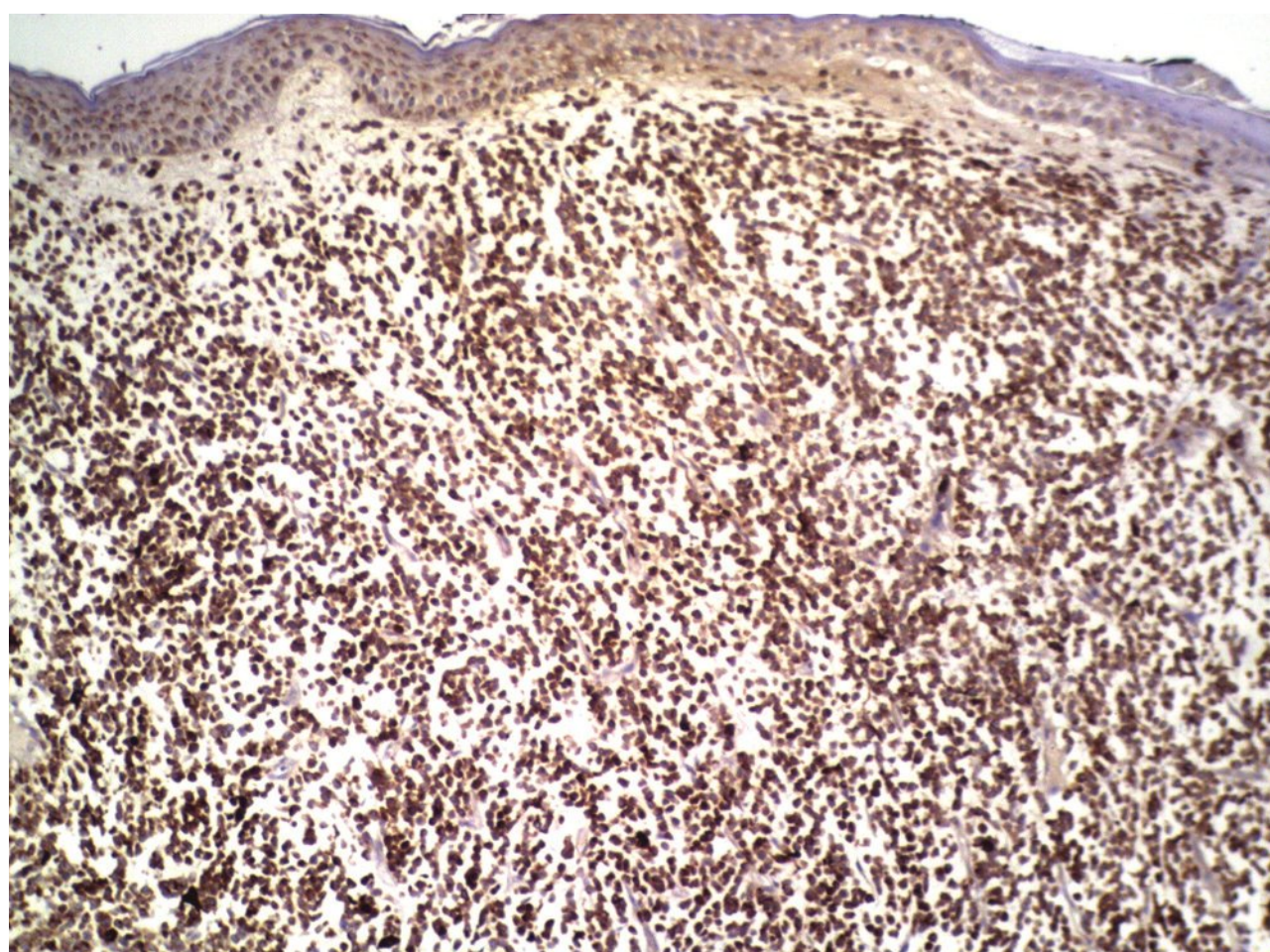


FIGURE 6.14.9 CD68 stain in the cutaneous monoblastic sarcoma. Immunoperoxidase, 10× magnification.

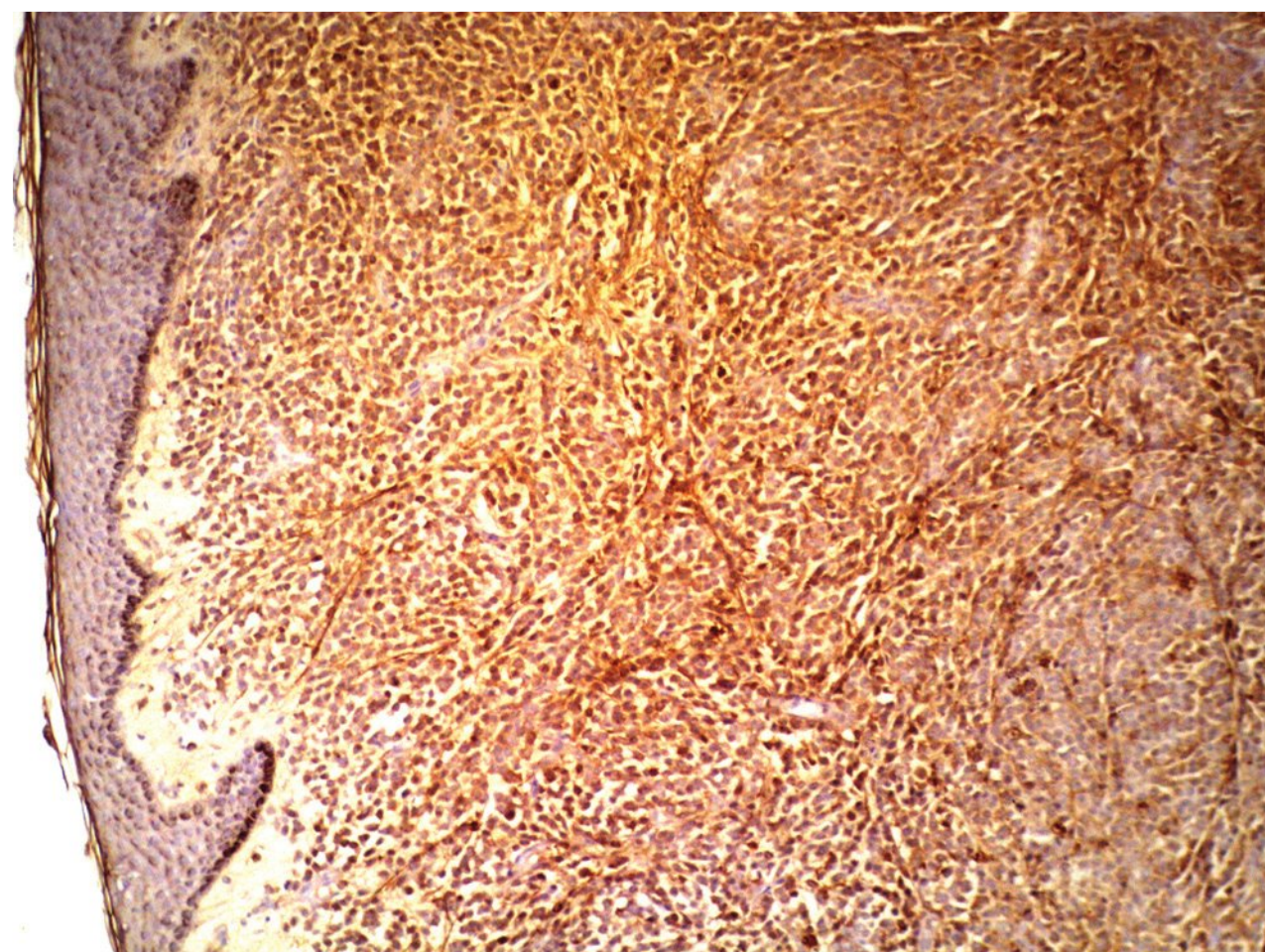


FIGURE 6.14.10 Lysozyme stain in the cutaneous monoblastic sarcoma. Immunoperoxidase, 10× magnification.

of these two markers in least-differentiated MS was confirmed by Hudock et al. (21).

According to the literature, the most sensitive markers appear to be lysozyme and CD43 (8,22–24). The presence of CD43 in MS has been further confirmed in several other studies (2,10,19,25,26). Although CD43 is a T-cell marker, the diagnosis of MS is valid when the so-called CD43 only pattern is present, in which other T-cell and B-cell markers are negative (23,27). However, in a study of 92 adult MS cases, the most commonly expressed marker was CD68 (KP1) (100%), followed by MPO (83.6%), CD117 (80.4%), CD99 (54.3%), CD68 (PG-M1) (51%), CD34 (43.4%), terminal deoxynucleotidyl transferase (TdT) (31.5%), CD56 (13%), CD61 (2.2%), CD30 (2.2%), and CD4 (1.1%) (28).

A speedy diagnosis can be made if tissue imprints are available. Cytochemical stains for MPO, chloroacetate esterase (for granulocytes), and α -naphthyl butyrate esterase (for monocytes) can be performed on such preparations.

Most of the comparative studies considered flow cytometry superior to immunohistochemistry for the diagnosis of MS. One of the reasons is that some markers (such as CD45) can be negative or weakly positive by immunohistochemical stains but strongly positive by flow cytometry in the same specimen (22,29). This is particularly true for cytospins, smears, and cell blocks, in which immunocytochemical or cytochemical stains are often difficult to interpret (30).

There are several markers that are not myelomonocytic markers but are useful for differential diagnosis. HLA-DR is helpful in identifying MS that is associated with acute promyelocytic leukemia, in which case HLA-DR should be very low or entirely absent. CD34, the stem cell marker, is present in MS cases (particularly the blastic type), but is negative for lymphomas (31). CD34, however, can be negative in well- and poorly differentiated types of MS (9). CD56, a neural cell adhesion molecule, can also be found in MS, and its expression usually predicts a poor prognosis (3). In fact, CD56 is probably one of the predisposing factors for the development of MS in patients with AMLs (32). The

blasts that express CD56 may bind to tissue expressing the same adhesion molecule, thus forming a solid tumor mass (33). Foci of plasmacytoid dendritic cells are occasionally seen in MS cases, which may express CD56 and CD123 (17).

A panel of CD45 together with T-cell and B-cell markers is a powerful screening tool to distinguish MS and lymphoma, as MS usually only expresses CD45 (but not T-cell and B-cell markers) (Fig. 6.14.11). However, rare cases of MS may demonstrate T-cell markers, such as CD45RO, (UCHL1), CD3, and CD7 (13,18–20). Less frequently, B-cell markers, such as CD20, Ki-B3, 4kB5, MB1, and LN2, have been reported in MS cases (13,19). These findings may represent nonspecific cross-reactivity, or, in some cases, they may represent a mixed lymphoid–myeloid phenotype (18,19,34). A tumor with a mixed lymphoid–myeloid phenotype is classified as acute leukemia of ambiguous lineage in the 2008 WHO classification (4).

Comparison of Flow Cytometry and Immunohistochemistry

Although most cases of MS are diagnosed by immunohistochemistry, flow cytometry is more helpful in some conditions. There are some antibodies that are available only for flow cytometry, such as CD13, CD11b, CD11c, CD14, and CD33. In MS of monocytic lineage, multiple monocytic antibodies (CD11b, CD11c, CD14, and CD64) should be used, because one or two of these markers can be negative in individual cases (22,35).

It is particularly critical to use flow cytometry in minimally differentiated AML (AML-M0) cases, because immunohistochemistry frequently fails to demonstrate diagnostic markers in those cases. The M0 case reported by Amin et al. (36) showed the absence of MPO, lysozyme, Sudan black B, specific and nonspecific esterase, and TdT. CD34 was demonstrated in rare cells. However, flow cytometry revealed HLA-DR, CD11c, CD13, CD15, CD34, and TdT. The report from Astall et al. (31) detected no CD15, chloroacetate esterase and lysozyme by immunohistochemistry. The

only diagnostic markers were CD45 and CD43. Flow cytometry in the same case, however, demonstrated CD7, CD13, CD33, CD34, and CD43. Finally, the report from Miyata et al. (37) revealed no immunohistochemical reactions to MPO, chloroacetate esterase, and lysozyme, but flow cytometry demonstrated CD7, CD13, CD33, CD41, and CD56.

The current case is also an M0 case, which showed the absence of CD34, MPO, chloroacetate esterase, and lysozyme by immunohistochemical stains, but flow cytometry detected high percentages of CD34 and CD117, as well as dual staining of CD33-CD13/CD7 in the peripheral blood, bone marrow, and lymph node biopsy; thus, a diagnosis of MS was established.

Molecular Genetics

The cytogenetic abnormalities most frequently associated with MS include t(8;21)(q22;q22), characteristic of M2; inv(16)(p13;q22) or t(16;16)(p13;q22), characteristic of M4 with eosinophilia; and t(9;11)(p21;q23), characteristic of M5 (3,38,39). On the other hand, AML with t(8;21) has a higher incidence of MS, particularly orbital MS (40). In fact, t(8;21) and the expression of CD56 may play a synergistic role in the development of MS (32,41). Translocation between chromosomes 8 and 21 results in the fusion of the AML1 gene on chromosome 21 to the eleven twenty-one (ETO) gene on chromosome 8. The novel chimeric gene (AML1/ETO) produces a transcript that may play a role in leukemic transformation (42). A case of coexistence of t(8;21)(AML1/ETO) and t(9;22)(BCR/ABL) was recently reported in an MS case (43). Yin et al. (44) suggested that the synergistic effect of BCR/ABL and AML/ETO might provide an additional growth advantage necessary for neoplastic transformation.

In contrast, pediatric patients with t(8;21) AML and MS usually have a good prognosis (39,45). In general, t(8;21) AML is associated with a younger age of onset, frequent splenomegaly, a high complete remission rate, and long relapse-free survival (33). No cytogenetic abnormalities have been identified in MS associated with myelodysplastic syndrome (MDS) (46).

A recent study by fluorescence in situ hybridization showed that 54.3% of MS cases had chromosomal aberrations (28). An array comparative genomic hybridization (CGH) study of seven MS cases revealed that all cases had genomic abnormalities and chromosome 8 was most commonly abnormal (47). Other reported cytogenetic aberrations include monosomy 7, trisomy 8, MLL rearrangement, trisomy 4, monosomy 16, 16q-, 5q-, 20q-, and trisomy 11 (4,28).

Current studies emphasized the presence of nucleophosmin 1 (NPM1) mutations in MS cases with an approximate incidence of 16% (4,48). This mutation can be predicted by the aberrant cytoplasmic expression of NPM protein as demonstrated by immunohistochemistry (48). The NPM-positive MS cases are usually associated with FAB M4 and M5 morphology and a normal karyotype (17). In acute myelogenous leukemia cases, NPM1 mutations in the absence of FLT3-ITD identify a subgroup of patients with favorable prognosis (48). The prognostic significance of the expression of NPM1 mutation in MS cases has not yet been established (48).

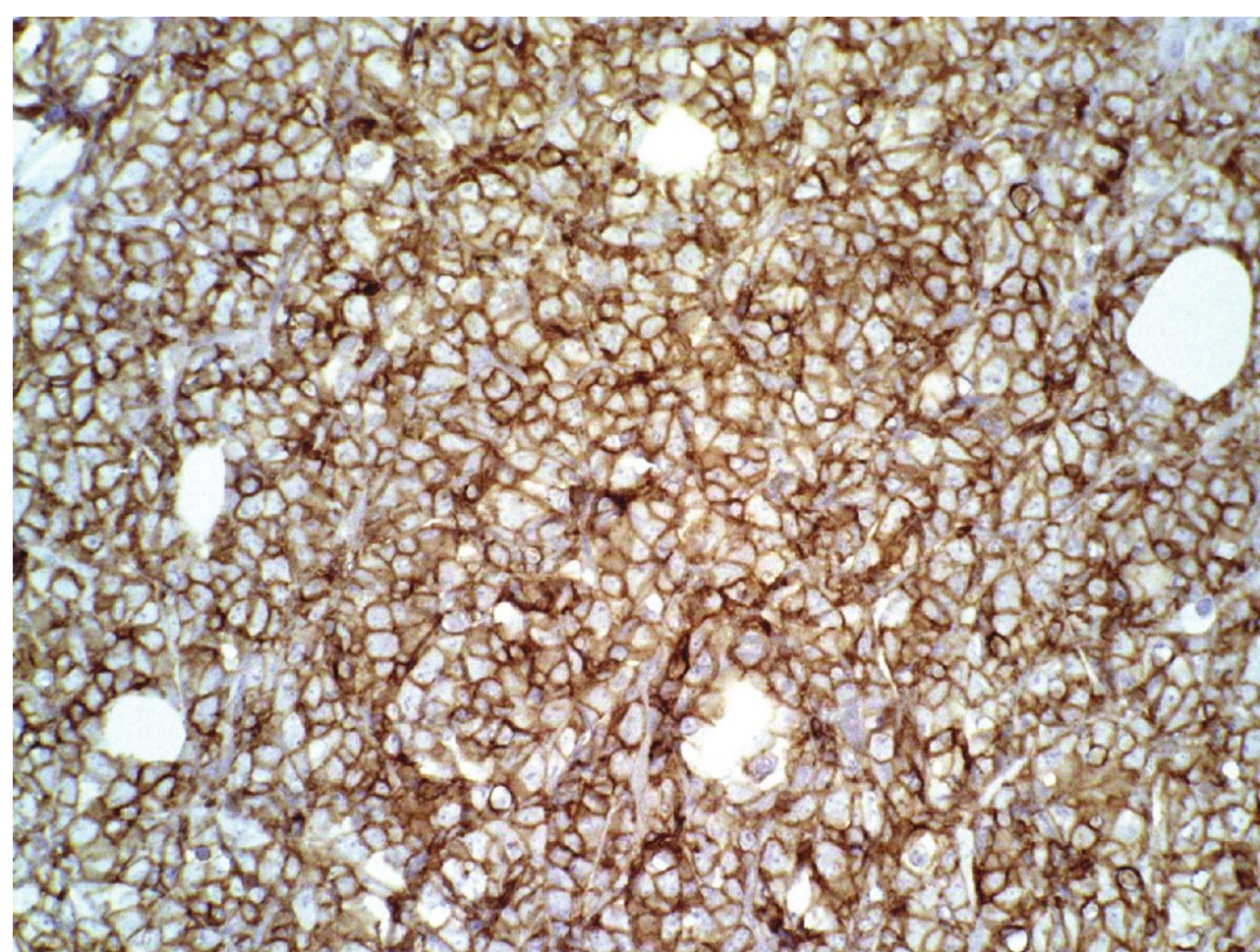


FIGURE 6.14.11 MS cells stains strongly with CD45, but negative with CD20 and CD3 (not shown). Immunoperoxidase, 20× magnification.



The salient features for laboratory diagnosis of MS are summarized in Table 6.14.2.

Clinical Manifestations

Most MS cases are associated with AML, CML, other types of myeloproliferative neoplasms, or MDS. These associated conditions can be present before, during, or after the occurrence of MS. In a few cases, the patient may never show features of leukemia or myelodysplasia. Those patients probably die of MS before leukemia or myelodysplasia starts to surface.

Up to 2004, approximately 800 cases of MS have been reported. According to a study series of 478 patients with myelogenous leukemias, more MS cases were associated with CML (4.5%) than AML (2.5%) (49). Less than 20 MS cases have been reported with MDS.

Among AML cases, the most commonly reported subtypes appear to be AML with maturation (AML-M2) and acute myelomonocytic leukemia with eosinophilia (AML-M4eo). Another source claimed that MS has a significantly increased incidence in M4 and M5 (3), whereas another report mentioned M1 and M2 as the most common leukemias developed after MS (31). However, as most case reports of MS did not subclassify the leukemia, the incidence of the leukemic subtypes cannot be accurately estimated.

Among MDS cases, chronic myelomonocytic leukemia and refractory anemia with excess blasts in transformation have a more frequent association with MS and with subsequent development of AML. Nevertheless, MDS-associated MS is not always a forerunner of AML (50). In general, MS is a predictor of poor prognosis in CML and MDS: Patients usually die in a few weeks after the discovery of MS. In CML, the occurrence of MS is frequently followed by blast crisis.

As mentioned before, MS is frequently misdiagnosed initially. In some MS cases, a correct diagnosis was not made even in subsequent recurrences of the tumor. One patient had a series of 11 episodes of MS in the subcutaneous tissue, lymph nodes, liver, and lumbosacral epidural space over 29 months. However, the recurrences in several episodes were still misdiagnosed for diseases other than MS (32).

The most frequently involved organs and/or tissues are soft tissue, periosteum and bone, lymph nodes, and skin. In female patients, ovaries and the breast are frequently involved (51). One patient had MS in the vagina, both breasts, and ovaries 6 months after the development of AML (52). In pediatric patients, orbital MS is the most frequent finding (39,53). However, many organ involvements were only discovered at autopsy. In autopsied cases, practically all major organs, except for the spleen, have been affected (3).

The age of MS has a wide range, varying from 1 week to 75 years. It has been seen mostly in the middle-aged male population, and less frequently in patients younger than 15 years. The mean age in several reported series is very close: 43 years reported by Eshghabadi et al. (54), 48 years by Neiman et al. (5), and 44 years by Friedman et al. (55).

The importance of making an accurate diagnosis of MS is due to its therapeutic implication. Patients who receive

TABLE 6.14.2

Salient Features for Laboratory Diagnosis of MS

- 1. Screening panel is composed of CD45, CD19 or CD20, and CD3. MS cases should be CD45+, CD19/CD20–, CD3–. Lymphoma cases should be CD45+ and either CD19/CD20+ or CD3+.
- 2. The standard flow cytometry panel for MS may include CD13, CD14, CD15, CD33, CD34, CD117, and MPO.
- 3. If monocytic sarcoma is suspected, CD11b, CD11c, CD4, and CD64 should be added.
- 4. Immunohistochemistry panel may include chloroacetate esterase (Leder stain), MPO, lysozyme, CD15, CD43, CD68, CD99, CD117, and CD163.
- 5. CD68 (KP-1), lysozyme, and CD43 are considered the most sensitive markers.
- 6. If monocytic sarcoma is suspected, a-naphthyl butyrate esterase should be added.
- 7. Common cytogenetic abnormalities include t(8;21)(q22;q22), inv(16), t(9;11)(p21;q23), and trisomy 8.
- 8. Immunohistochemical staining for nucleophosmin or detection of NPM 1 mutation is recommended but not required.

MS, myeloid sarcoma.

antileukemic therapy with or without local radiation therapy usually have a long remission, whereas other treatments, such as surgical, radiation, and antilymphoma therapy, usually show no effects. Patients who were treated with antileukemic therapy within 4 months from the initial diagnosis of MS achieved complete remission of both MS and leukemia (6). In contrast, patients initially treated for lymphoma usually failed to respond to the subsequent correct treatment and had a dismal prognosis (20). Therefore, the current opinion advocates treating MS as AML, even in the absence of leukemic manifestations (7,20,38,54,55).

REFERENCES

1. Brunning RD, Matutes E, Flandrin G, et al. Acute myeloid leukaemia not otherwise categorized. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001:91–107.

2. Dabbagh V, Browne G, Parapia LA, et al. Granulocytic sarcoma of the rectum: a rare complication of myelodysplasia. J Clin Pathol. 1999;52:865–866.

3. Byrd JC, Edenfield J, Shields DJ, et al. Extramedullary myeloid cell tumors in acute nonlymphocytic leukemia: a clinical review. J Clin Oncol. 1995;13:1800–1816.

4. Pileri SA, Orazi A, Falini B. Myeloid sarcoma. In: Swerdlow SH, Campo E, Harris NL, et al. eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:140–141.

5. Neiman RS, Barcos M, Berard C, et al. Granulocytic sarcoma: a clinicopathologic study of 61 biopsied cases. *Cancer*. 1981;48:1426–1437.
6. Meiss JM, Butler JJ, Osborne BM, et al. Granulocytic sarcoma in nonleukemic patients. *Cancer*. 1986;58:2697–2709.
7. Breccia M, Mandelli F, Petti MC, et al. Clinico-pathological characteristics of myeloid sarcoma at diagnosis and during follow-up: report of 12 cases from a single institution. *Leuk Res*. 2004;28:1165–1169.
8. Alexiev BA, Wang W, Ning Y, et al. Myeloid sarcomas: a histologic, immunohistochemical, and cytogenetic study. *Diagn Pathol*. 2007;2:42–49.
9. Traweek ST, Arber DA, Rappaport H, et al. Extramedullary myeloid cell tumors: an immunohistochemical and morphologic study of 28 cases. *Am J Surg Pathol*. 1993;17:1011–1019.
10. Audouin J, Comperat E, Le Tourneau A, et al. Myeloid sarcoma: clinical and morphologic criteria useful for diagnosis. *Int J Surg Pathol*. 2003;11:271–282.
11. Strauchen JA. Sarcomatoid neoplasm of monocytic lineage. *Am J Surg Pathol*. 1991;15:1206–1208.
12. Carmichael GP, Lee YT. Granulocytic sarcoma simulating “non-secretory” multiple myeloma. *Hum Pathol*. 1977;8:697–700.
13. Davey FR, Olsen S, Kurec AS, et al. The immunophenotyping of extramedullary myeloid cell tumors in paraffin-embedded tissue sections. *Am J Surg Pathol*. 1988;12:699–707.
14. Roth MJ, Medeiros J, Elenitoba-Johnson K, et al. Extramedullary myeloid cell tumors: an immunohistochemical study of 29 cases using routinely fixed and processed paraffin-embedded tissue sections. *Arch Pathol Lab Med*. 1995;119:790–798.
15. Zhang PJ, Barcos M, Stewart CC, et al. Immunoreactivity of MIC2 (CD99) in acute myelogenous leukemia and related disease. *Mod Pathol*. 2000;13:452–458.
16. Chen J, Yanuck RR, Abbondanzo SL, et al. C-kit (CD117) reactivity in extramedullary myeloid/granulocytic sarcoma. *Arch Pathol Lab Med*. 2001;125:1448–1452.
17. Campidelli C, Agostinelli C, Stitson R, et al. Myeloid sarcoma: Extramedullary manifestation of myeloid disorders. *Am J Clin Pathol*. 2009;132:426–437.
18. Furebring-Freden M, Martinsson U, Sundstrom C. Myelosarcoma without acute leukemia. Immunohistochemical and clinicopathologic characterization of eight cases. *Histopathology*. 1990;16:243–250.
19. Horny HP, Campbell M, Steinke B, et al. Acute myeloid leukemia: immunohistologic findings in paraffin-embedded bone marrow biopsy specimens. *Hum Pathol*. 1990;21:648–655.
20. Fellbaum C, Hansmann ML. Immunohistochemical differential diagnosis of granulocytic sarcoma and malignant lymphomas on formalin-fixed material. *Virchows Arch A Pathol Anat Histopathol*. 1990;416:351–355.
21. Hudock J, Chatten J, Miettinen M. Immunohistochemical evaluation of myeloid leukemia infiltrates (granulocytic sarcoma) in formaldehyde-fixed, paraffin-embedded tissue. *Am J Clin Pathol*. 1994;102:55–60.
22. Dunphy CH, Martin DS. Extramedullary tumor of monoblasts in the central nervous system: presenting feature of simultaneous bone marrow involvement by acute monocytic leukemia. *Arch Pathol Lab Med*. 1999;123:327–331.
23. Menasce LP, Banerjee SS, Beckert E, et al. Extra-medullary myeloid tumour (granulocytic sarcoma) is often misdiagnosed: a study of 26 cases. *Histopathology*. 1999;34:391–398.
24. McCluggage WG, Boyd HK, Jones FD, et al. Mediastinal granulocytic sarcoma: a report of two cases. *Arch Pathol Lab Med*. 1998;122:545–547.
25. Valbuena JR, Admirand JK, Gualko G, et al. Myeloid sarcoma involving the breast. *Arch Pathol Lab Med*. 2005;129:32–38.
26. Tao J, Wu M, Fuchs A, et al. Fine-needle aspiration of granulocytic sarcomas: a morphologic and immunophenotypic study of seven cases. *Ann Diagn Pathol*. 2000;4:17–22.
27. Segal GH, Stoler MH, Tubs R. The “CD43 only” phenotype: an aberrant, nonspecific immunophenotype requiring comprehensive analysis for lineage resolution. *Am J Clin Pathol*. 1992;97:861–865.
28. Pileri SA, Ascani S, Cox, et al. Myeloid sarcoma: clinicopathologic, phenotypic and cytogenetic analysis of 92 adult patients. *Leukemia*. 2007;21:340–350.
29. Quintanilla-Martinez L, Zukerberg LR, Ferry JA, et al. Extramedullary tumors of lymphoid or myeloid blasts. The role of immunohistology in diagnosis and classification. *Am J Clin Pathol*. 1995;104:431–433.
30. Liu K, Mann KP, Garst JL, et al. Diagnosis of posttransplant granulocytic sarcoma by fine-needle aspiration cytology and flow cytometry. *Diagn Cytopathol*. 1999;20:85–89.
31. Astall E, Yarranthon H, Arnl J, et al. Granulocytic sarcoma preceding AML M0 and the diagnostic value of CD34. *J Clin Pathol*. 1999;52:705–707.
32. Byrd JC, Weiss RB. Recurrent granulocytic sarcoma. An unusual variation of acute myelogenous leukemia associated with 8;21 chromosomal translocation and blast expression of the neural cell adhesion molecule. *Cancer*. 1994;73:2107–2112.
33. Tallman MS, Hakimian D, Shaw JM, et al. Granulocytic sarcoma is associated with 8;21 translocation in acute myeloid leukemia. *J Clin Oncol*. 1993;11:690–697.
34. Hossain D, Weisberger J, Sreekantaiah C, et al. Biphenotypic (mixed myeloid/T-cell) extramedullary myeloid cell tumor. *Leuk Lymphoma*. 1999;33:399–402.
35. Lauritzen AF, Delsol G, Hansen NE, et al. Histiocytic sarcomas and monoblastic leukemias: a clinical, histologic and immunophenotypic study. *Am J Clin Pathol*. 1994;102:45–54.
36. Amin KS, Ehsan A, McGuff HS, et al. Minimally differentiated acute myelogenous leukemia (AML-M0) granulocytic sarcoma presenting in the oral cavity. *Oral Oncol*. 2002;38:516–519.
37. Miyata A, Fujii S, Kijuchi T, et al. Acute myelocytic leukemia (M0) in an elderly patient with relapsed granulocytic sarcoma (M7) of bone during the second period of complete remission 5 years after onset [in Japanese]. *Nippon Ronen Igakkai Zasshi (Jpn J Geriatrics)*. 2003;4:507–513.
38. Hutchison RE, Kurec AS, Davey FR. Granulocytic sarcoma. *Clin Lab Med*. 1990;10:889–901.
39. Brown NP, Rowe D, Reid MM. Granulocytic sarcoma with translocation (9;11)(p22;q23): two cases. *Cancer Genet Cytogenet*. 1997;96:115–117.
40. Schwyzer R, Sherman GG, Cohn RJ, et al. Granulocytic sarcoma in children with acute myeloblastic leukemia and t(8;21). *Med Pediatr Oncol*. 1998;31:144–149.
41. Krishnan K, Ross CW, Adams PT, et al. Neural cell-adhesion molecule (CD56)-positive, t(8;21) acute myeloid leukemia (AML-M2) and granulocytic sarcoma. *Ann Hematol*. 1994;69:321–323.
42. Caligiuri MA, Strout MP, Gilliland DG. Molecular biology of acute myeloid leukemia. *Semin Oncol*. 1997;24:32–44.
43. Jondle DM, Sun T, Woods JE, et al. The role of flow cytometry in the diagnosis of myeloid sarcoma. Unpublished data.

44. Yin CC, Medeiros LJ, Glassman AB, et al. t(8;21)(q22;q23) in blast phase of chronic myelogenous leukemia. *Am J Clin Pathol*. 2004;121:836–842.
45. Felice MS, Zubizarreta PA, Alfaro EM, et al. Good outcome of children with acute myeloid leukemia and t(8;21)(q22;q22), even when associated with granulocytic sarcoma: a report from a single institute in Argentina. *Cancer*. 2000;88:1939–1944.
46. List A, Gonzalez-Osete G, Kummet T, et al. Granulocytic sarcoma in myelodysplastic syndromes: clinical marker of disease acceleration. *Am J Med*. 1991;90:274–276.
47. Deeb G, Baer MR, Gaile DP, et al. Genomic profiling of myeloid sarcoma by array comparative genomic hybridization. *Genes Chrom Cancer*. 2005;44:373–383.
48. Falini B, Lenze D, Hasserjian R, et al. Cytoplasmic mutated nucleophosmin (NPM) defines the molecular status of a significant fraction of myeloid sarcomas. *Leukemia*. 2007;21:1566–1570.
49. Muss HB, Maloney WC. Chloroma and other myeloblastic tumors. *Blood*. 1973;42:721–728.
50. Byrd JC, Edenfield WJ, Dow NS, et al. Extramedullary myeloid cell tumors in myelodysplastic syndromes: not a true indication of impending acute myeloid leukemia. *Leuk Lymphoma*. 1996;21:153–159.
51. Liu PI, Ishimaru T, McGregor DH, et al. Autopsy study of granulocytic sarcoma (chloroma) in patients with myelogenous leukemia. Hiroshima-Nagasaki 1949–1969. *Cancer*. 1973;31:948–955.
52. Gralnick HR, Dittmar K. Development of myeloblastoma with massive breast and ovarian involvement during remission in acute leukemia. *Cancer*. 1969;24:746–749.
53. Stockl FA, Dolmetsch AM, Saornil MA, et al. Orbital granulocytic sarcoma. *Br J Ophthalmol*. 1997;81:1084–1088.
54. Eshghabadi M, Shojania AM, Carr I. Isolated granulocytic sarcoma: report of a case and review of the literature. *J Clin Oncol*. 1986;4:912–917.
55. Friedman HD, Adelson MD, Elder RC, et al. Granulocytic sarcoma of the uterine cervix—literature review of granulocytic sarcoma of the female genital tract. *Gynecol Oncol*. 1992;46:128–137.

CASE 15

Blastic Plasmacytoid Dendritic Cell Neoplasm

CASE HISTORY

A 39-year-old man presented with progressive skin lesions on his trunk associated with constitutional symptoms for 6 months. He had arthralgias, bone pain in the ankles and wrists, fatigue, fever, and a 20-lb weight loss over a period of 6 months. He was treated with a short course of antibiotics without benefit. Physical examination revealed diffuse subcutaneous nodules of varying sizes over his trunk with the largest nodule measuring 4 cm. There was no lymphadenopathy, and the liver and spleen were not palpable. Hematology workup showed no cytopenia or leukocytosis.

A skin biopsy showed extensive tumor cell infiltration in the dermis and subcutis without epidermic involvement (Figs. 6.15.1 and 6.15.2). The bone marrow biopsy also revealed infiltration by tumor cells of the same morphology as those seen in the skin (Figs. 6.15.3 and 6.15.4). The patient received three cycles of induction chemotherapy with an acute lymphoblastic leukemia regimen. As a result, he had a complete resolution of his skin lesions and systemic symptoms. A second bone marrow biopsy was found to be negative for leukemic cells by both morphology and flow cytometric immunophenotyping. He subsequently received an autologous stem cell transplant. However, a third bone marrow biopsy 8 months later showed evidence of relapse. A new chemotherapy regimen was started in anticipation of a second autologous bone marrow transplantation.

IMMUNOHISTOCHEMISTRY

The skin biopsy showed that the tumor cells were positive for CD4, CD56, and CD68, but were negative for CD3, CD5, CD8, CD20, myeloperoxidase, and lysozyme.

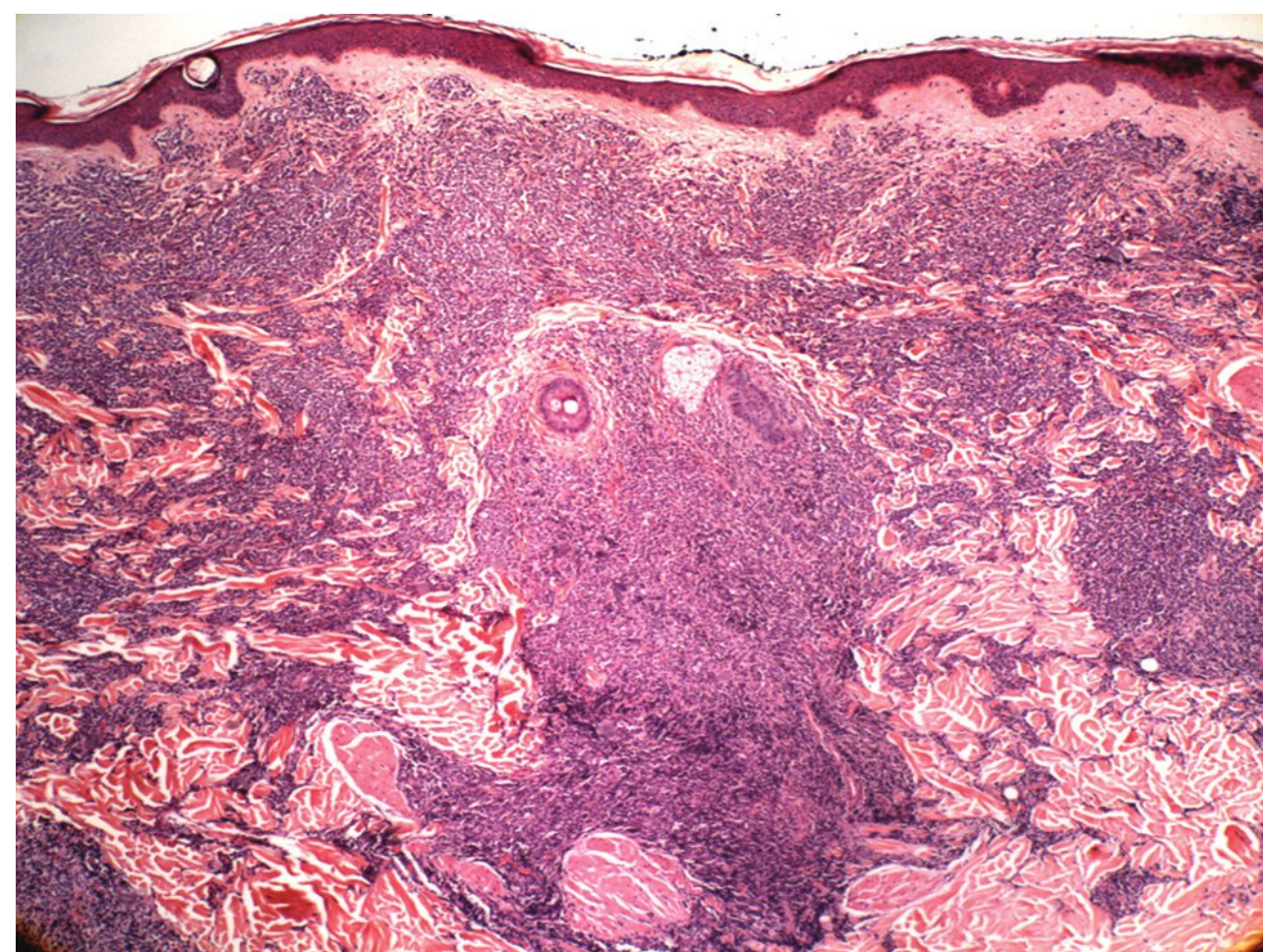


FIGURE 6.15.1 Skin biopsy shows extensive lymphoid infiltration of the entire dermis. Note a Grenz zone is present between the epidermis and the infiltrate. No epidermal involvement is demonstrated. H&E, $\times 4$.

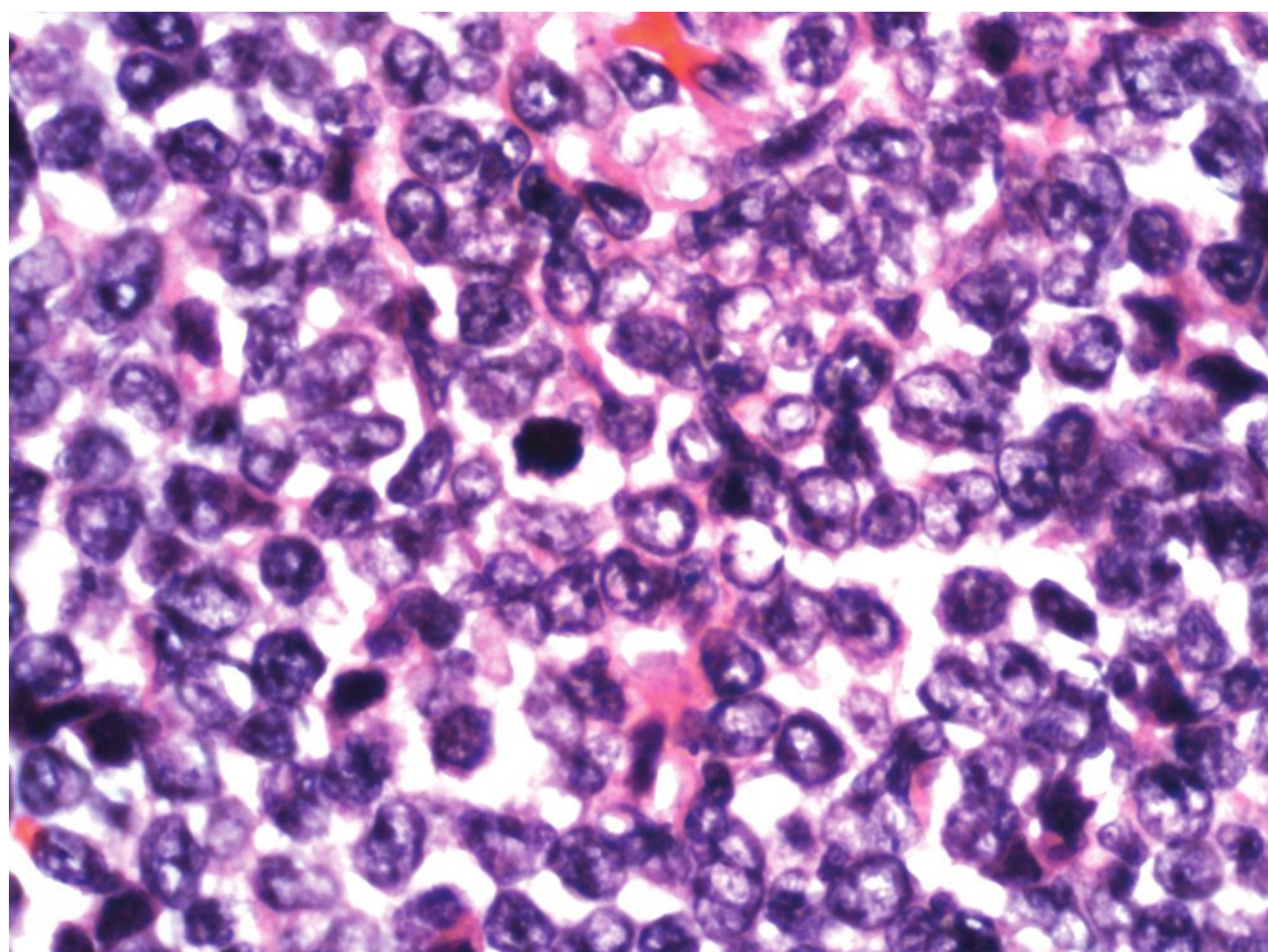


FIGURE 6.15.2 High magnification of the skin biopsy shows immature nuclear chromatin pattern with occasional nucleoli. H&E, ×100.

FLOW CYTOMETRY

The skin biopsy showed a gated population with positive reactions to CD2, cytoplasmic CD3, CD4, CD7, CD45, CD56, and HLA-DR, but negative reactions to surface CD3, CD8, CD10, CD13, CD19, CD33, CD34, CD117, terminal deoxynucleotidyl transferase (TdT), and myeloperoxidase.

The bone marrow biopsy showed positive reactions to CD2, CD4, CD7, CD13, CD33, and CD56, but negative reactions to CD3, CD8, CD11c, CD14, CD19, CD20, CD57, k, l, and TdT.

MOLECULAR GENETICS

T-cell receptor gene rearrangement analysis of bone marrow showed a germline pattern.

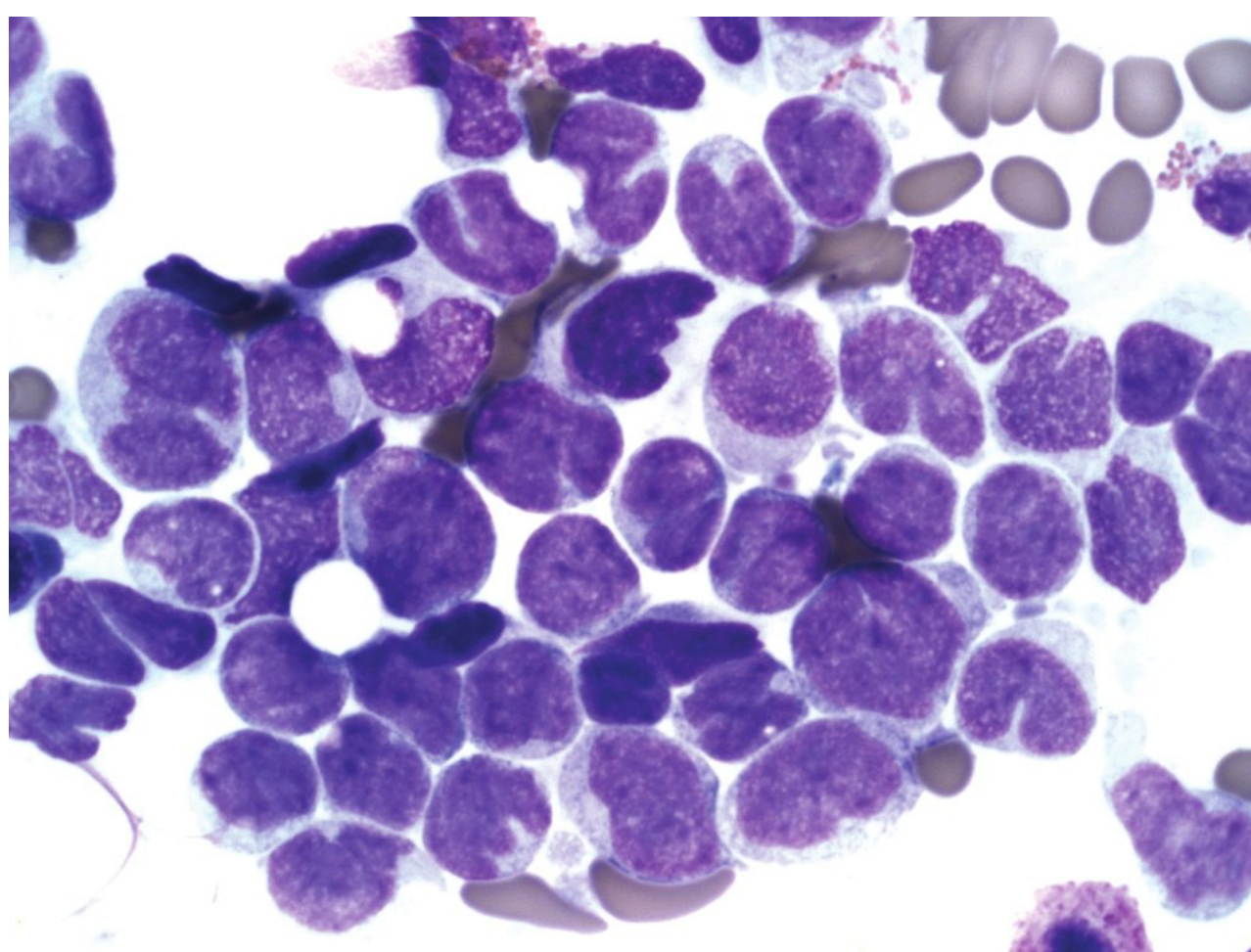


FIGURE 6.15.3 Bone marrow aspirate shows blast-like cells with irregular nuclei and immature chromatin pattern. Wright–Giemsa, ×100.

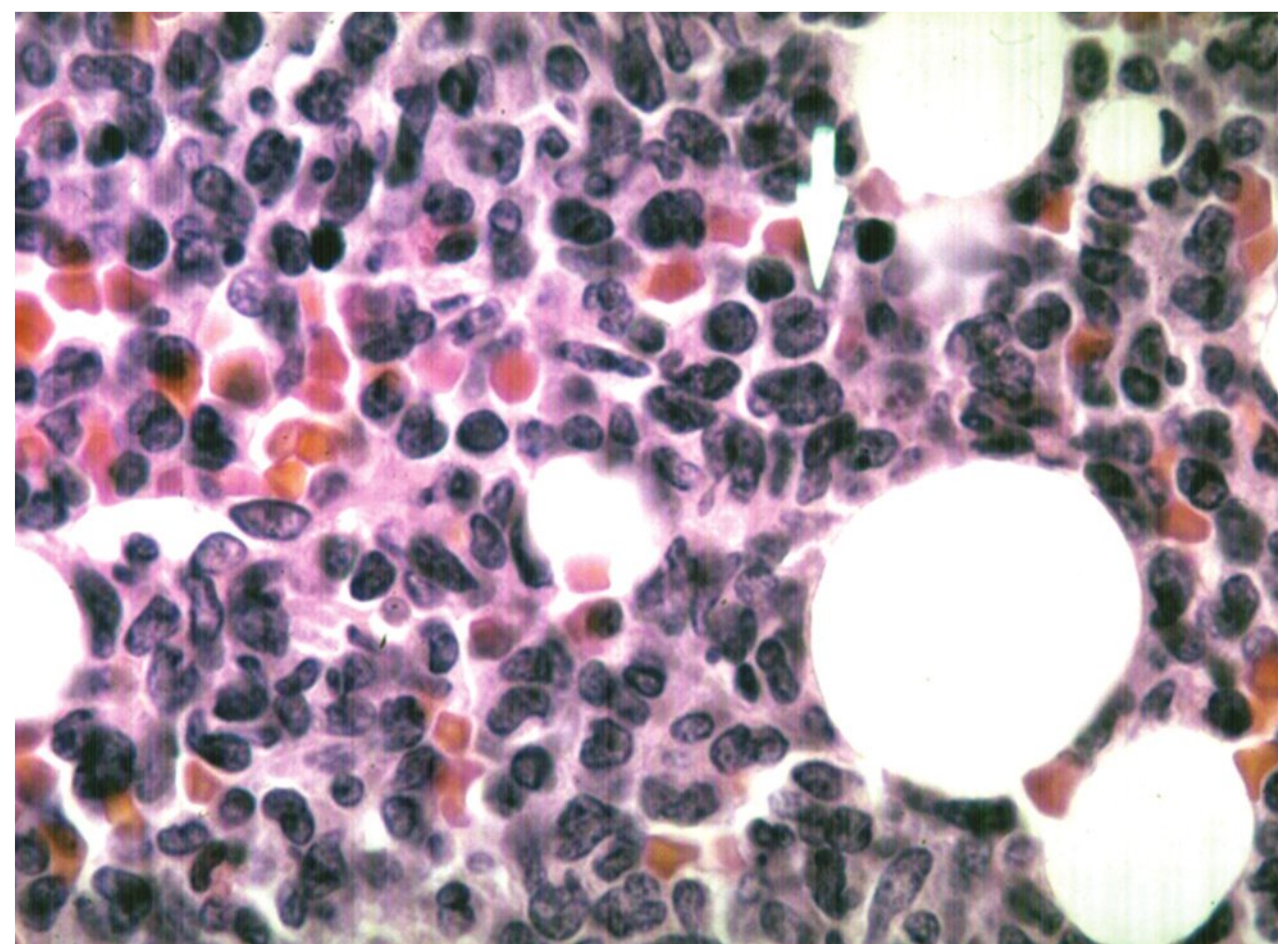


FIGURE 6.15.4 Bone marrow biopsy shows total replacement of normal hematopoietic cells by leukemic components. Note a few bilobed cells (arrow). H&E, ×100. (From Sun T, *Atlas of Hematologic Neoplasms*, Dordrecht, Germany, Springer, 2009, with permission)

DISCUSSION

In 1995, Brody et al. (1) described a case of purportedly CD56+ natural killer (NK)-cell leukemia, which differed from the classical NK-cell neoplasm in its agranular morphology, CD4+ (instead of CD8+) immunophenotype, and an initial skin presentation. Different nomenclature were later applied to this neoplasm in subsequently reported cases, such as cutaneous agranular CD2−/CD4+/CD56+ lymphoma, agranular CD4+/CD56+ blastic NK leukemia/lymphoma, CD4+, CD56+ acute leukemia, and CD56+ TdT+ blastic NK-cell tumor of skin, but its NK-cell nature was never challenged (2). The World Health Organization (WHO) classification designated this tumor as blastic NK-cell lymphoma in 2001 (3). It was the Petrella's group that first questioned about the cellular origin of this tumor and coined the uncommitted descriptive term of agranular CD4+ CD56+ hematodermic neoplasm (4–6). The term CD4+/CD56+ hematodermic neoplasm was adopted by the WHO/European Organization for Research and Treatment of Cancer (EORTC) classification for cutaneous lymphomas in 2005 (7).

Recent immunophenotypic and functional characterization of normal plasmacytoid dendritic cell (PDC) and the CD4+ CD56+ hematodermic neoplasm has finally provided evidence that this tumor actually derives from PDC. Immunologically, this tumor shares with PDC the markers of CD4, CD123 (IL-3a receptor), T-cell leukemia/lymphoma 1 (TCL1), cutaneous lymphocyte-associated antigen (CLA), blood dendritic cell antigen 2 (BDCA2)/CD303, BDCA4/CD304, and CD2-associated adaptor protein (CD2AP) (8,9). A minor subset of PDC also expresses CD56 (10). Upon in vitro stimulation with IL-3 and CD40L, these tumor cells may differentiate into cells with dendritic processes. In addition, these tumor cells can also produce interferon-alpha (IFN-α) that is characteristic of PDC (8).

Given all these evidence, most current studies support the PDC origin of the CD4+, CD56+ hematodermic neoplasm, and the 2008 WHO classification designates it as blastic plasmacytoid dendritic cell neoplasm (BPDCN) (11).

Morphology

The morphologic description of the BPDCN cells in the literature is greatly variable, but one consistent feature is that the tumor cells are blast-like with immature chromatin and with or without the presence of nucleoli (6–8,11–13). The cell size ranges from small to large, but medium-sized cells are most frequently encountered. The nuclei are usually round or oval, but irregular configuration (notched, folded, or bilobed) has also been reported. The cytoplasm can be scanty or abundant with gray–blue color. The specific features described in several studies are the presence of peripheral microvacuoles in the cytoplasm (pearl necklace appearance) and pseudopod-like extensions, recapitulating the pinocytotic process in normal PDCs (6,12,13). Cytoplasmic granules are characteristically absent. Mitosis may be present but is usually not prominent.

In the skin biopsy, dermis is extensively infiltrated with frequent extension into the subcutis, while epidermis is usually not involved. In the early lesion, there is a perivascular and periadnexal infiltration, but later lesion may show destruction of the skin appendages (6). The degree of infiltration in the bone marrow depends on the stage of the disease. In the late stage, the normal hematopoietic cells can be largely replaced. Residual hematopoietic cells, especially megakaryocytes, may exhibit dysplasia (6,13). Lymph node involvement often presents as leukemic-type infiltration in the interfollicular area, but effacement of nodal architecture may be demonstrated in the later stage.

Immunophenotype

The immunophenotype for initial diagnosis of BPDCN is the CD4+, CD56+, cell lineage–negative profile. Myelomonocytic markers (CD13, CD33, CD11c, CD14, CD64, myeloperoxidase, chloroacetate esterase, butyrate esterase, and lysozyme), B-cell (CD19, CD20, CD79a, PAX5) and T-cell (CD1a, CD2, CD3, CD5, CD7, and CD8) lineage–associated markers, NK cell–associated antigens (CD16, CD57, T-cell intracellular antigen 1, and perforin), progenitor cell markers (CD34 and CD117), and lymphoid activation markers (CD25, CD30, and CD71) are usually absent (8,15). CD21, CD35, CD36, CD10, S100, and Epstein–Barr virus antigen are also not present in this tumor. However, a few lineage markers may be present occasionally, such as CD2, cytoplasmic CD3, CD5, CD7, CD10, CD20, CD22, CD33, and CD117 (2,8,12,15,16). One study found that CD22 is present in normal PDCs and basophils (17). Terminal nucleotidyl transferase (TdT) may be expressed in a primitive subset of BPDCN and is associated with an aggressive clinical course (2,18). There is also an inverse correlation between TdT and BDCA2 expression (19). CD101, a marker expressed by a major dendritic cell subset, is also frequently positive on BPDCN cells (20). Other nonspecific markers including CD45, CD45RA, CD43, HLA-DR, and CD68 are frequently present. However, it is most important to demonstrate those PDC-specific markers for a definitive diagnosis.

There are probably many subtypes of dendritic cells due to their great variation in immunophenotype, therefore, several different classifications are existent. However, the generally recognized subsets are dendritic cell type 1 (DC1), also called myeloid dendritic cells, and dendritic cell type 2 (DC2), also known as plasmacytoid or lymphoid dendritic cells (PDCs). DC1 expresses BDCA3, CD11c, CD13, CD33 but not CD123. DC2, on the other hand, expresses BDCA2, BDCA4, and CD123 but not CD11c, CD13, and CD33 (2,15,21). However, DC2 may be converted into DC1 and myeloid cells *in vivo* and *in vitro* under different stimulations (2). In addition, DC2 can be generated from macrophage colony stimulating factor–positive CD34+ progenitors (2). DC1 leukemia or acute myeloid dendritic cell leukemia is an exceedingly rare disease, which also expresses a CD4+ CD56+ immunophenotype with initial skin involvement. However, the tumor cells also show many myeloid markers, such as CD13, CD14, CD15, CD33, myeloperoxidase, lysozyme, as well as the DC1 marker (BDCA3) (21).

The current case showed an immunophenotype characteristic of BPDCN in the skin, but the bone marrow biopsy was atypical in the presence of myeloid marker (22). However, the absence of a leukemic phase in this patient was not in favor of the diagnosis of myelomonocytic leukemia.

In the earlier literature, PDC was also called T-associated plasma cell, plasmacytoid T cell, and plasmacytoid monocytes (2,8,9). These cells can be present in nonmalignant conditions, such as Kikuchi lymphadenitis, Castleman disease, lupus erythematosus, and lichen planus (9). PDC nodules are frequently present in the lymph nodes, skin, spleen, or bone marrow in cases of chronic myelomonocytic leukemia and other myeloid neoplasms with monocytic differentiation (23).

The PDC specific markers, including CD123, BDCA2, BDCA4, CD2AP, TCL1, and CLA, are most useful in the differential diagnosis. The IFN- α produced by BPDCN can be identified by antibodies for myxovirus A (MxA) protein, as a surrogate marker (15). Among all these markers, BDCA2 and CD2AP seem to be most specific (8,9). However, some of these markers may also be present in other hematologic neoplasms. For instance, CD123 can be demonstrated in acute basophilic leukemia, Langerhans cell histiocytosis, histiocytic sarcoma, and hairy cell leukemia (8,24). TCL1 can be present in T-cell prolymphocytic leukemia, T-cell acute lymphoblastic leukemia, adult T-cell lymphoma/leukemia, and some B-cell lymphomas (25,26). CLA is also detected in leukemia cutis secondary to acute myeloid leukemia or chronic myelomonocytic leukemia (26). Among the blood dendritic cell antigens, BDCA2 is more specific than BDCA4 for the diagnosis of BPDCN; BDCA4 was demonstrated in 12% of nondendritic cell acute lymphoid or myeloid leukemias, while BDCA2 was not detected in all 113 nondendritic cell leukemias (27).

Garnache-Ottou et al. (27) proposed a scoring system for the differential diagnosis of BPDCN. The presence of a profile of CD4+, CD56+, CD11c-, MPO-, cCD79a-, cCD3- is counted as one point. The presence of CD123 or BDCA4 also scores one point. The presence of BDCA2 scores two points. With the staining of all the above markers, BPDCN

usually scores more than 3 points, but nondendritic cell tumors score 2 points or below (27).

The major differential diagnosis includes cutaneous T-cell lymphoma, extranodal NK/T-cell lymphoma, adult T-cell leukemia/lymphoma, and leukemia cutis. To distinguish them morphologically and clinically can be a challenge, but a comprehensive immunophenotyping usually makes the distinction. Cutaneous T-cell lymphoma may share with BPDCN the expression of CD4 and CLA, but not CD56, CD123, and BDCA2. NK cells are similar to BPDCN in expressing CD56 and in absence of surface CD3. However, NK tumors are usually positive for EBV and cytotoxic proteins but do not express all the PDC markers and CD4. Adult T-cell leukemia/lymphoma shares no common antigens with BPDCN except for CD4. The most difficult differential diagnosis is cutaneous infiltration by myelomonocytic or monocytic leukemia, which may express CD4, CD56, CD68 and in some cases, CD123. Leukemia cutis also shows negative reactions to T- and B-cell lineage antigens. However, myelomonocytic/monocytic leukemia always expresses myelomonocytic markers but not BDCA2 and CD2AP. The distinction between BPDCN and cutaneous myelomonocytic leukemia is listed in Table 6.15.1.

Comparison between Flow Cytometry and Immunohistochemistry

Flow cytometry can screen a large number of lineage specific antigens and is most useful for initial diagnosis of BPDCN. Besides CD4/CD56, flow cytometry is also able to demonstrate specific markers, including CD123, BDCA2, BDCA3, and BDCA4. Therefore, some studies highly recommended the use of flow cytometry for the diagnosis of this entity. Tsagarakis et al. (16) advocate the use of CD45/side-scatter (SC) gating to separate BPDCN from CD4/CD56 positive leukemia, particularly myelomonocytic leukemia. The former is generally located in the CD45/SC low cluster, while the latter is in CD45/SC high cluster. BPDCN can then be identified by a group of positive markers (CD4, CD56, CD123, BDCA2, BDCA4, CD45RA, and HLA-DR) and a group of negative markers (MPO, CD3, cCD3, CD14, CD19, CD64, cCD79a, and MPO) (16). The myelomonocytic leukemia can be identified at least by three positive markers (CD64, MPO, and lysozyme) and two negative markers (BDCA2 and CD45RA) (16).

Immunohistochemical stains are more frequently used than flow cytometry because most specimens are probably fixed before this diagnosis is considered. In addition, a direct morphologic correlation with the immunohistochemical markers makes the pathologists more confident in the diagnosis. The specific markers that can be used in immunohistochemical stains include CD4, CD56, CD123, BDCA2 CD2AP, CLA, and TCL1 (9,16).

Molecular Genetics

Cytogenetic abnormalities have been identified in about two thirds of BPDCN patients, but specific karyotypic aberrations have not been found. Most of the anomalies are of complex karyotypes. However, six major recurrent chromosomal targets have been recognized, which include 5q (72%), 12p (64%), 13q (64%), 6q (50%), 15q (43%), and loss of chromosome 9 (28%) (28).

TABLE 6.15.1

Major Distinction between BPDCN and Cutaneous Myelomonocytic Leukemia

	BPDCN	CML
CD4	+	+
CD56	+	+
CD123	+	±
BDCA2	+	-
BDCA4	+	-
CD2AP	+	-
TCL1	+	-
CLA	+	+
CD68	+	+
CD45RA	+	-
CD11c	-	+
CD13	-	+
CD33	-	+
CD64	-	+
Myeloperoxidase	-	+
Lysozyme	-	+
B-cell markers	-	-
TCR gene rearrangement	-	-

BDCA, blood dendritic cell antigen; BPDCN, blastic plasmacytoid dendritic cell neoplasm; CD2AP, CD2-associated adaptor protein; CLA, cutaneous lymphocyte-associated antigen; CML, cutaneous myelomonocytic leukemia; TCL1, T-cell leukemia/lymphoma 1; TCR, T-cell receptor.

The integrated genomic analysis using gene expression profiling and array-based comparative genomic hybridization conducted by Dijkman et al. (29) clarified several points. First, this study identified high expression of various PDC-related genes in BPDCN cases, substantiating the cell origin of this tumor. Second, it demonstrated distinct gene expression profiles and distinct patterns of chromosomal aberrations between BPDCN and cutaneous myelomonocytic leukemia. Thirdly, BPDCN was characterized by recurrent deletion of regions on chromosomes 4, 9, and 13 that contain several tumor suppressor genes with diminished expression (Rb1, LATS2). Fourthly, elevation of the oncogenes HES6RUNX2 and FLT3 was found but was not associated with genomic amplification.

Using high-resolution array comparative genomic hybridization and quantitative multiplex polymerase chain reaction of short fluorescent fragments, Jardin et al. (30) demonstrated losses of multiple genes involved in the G1/S transition. The most common ones include deletions of chromosome 9 (containing CDKN2A and CDKN2B), 13q (containing the RB1 locus), 17p (containing TP53), and 12p (containing CDJB1B and ET1/6). With quantitative reverse transcription-polymerase chain reaction, Garnache-Ottou et al. (27) showed high levels

TABLE 6.15.2

Salient Features for Laboratory Diagnosis of PBDCN

- 1. Basic immunophenotype: CD4+ CD56+ cell lineage negative profile.
- 2. Specific markers: CD123, BDCA2, BDCA4, CD2AP, TCL1, and CLA.
- 3. INF- α product can be identified by antibodies for myxovirus A.
- 4. Negative T-cell markers: CD2, CD3, CD5, CD7, CD8.
- 5. Negative B-cell markers: CD19, CD20, CD79a, PAX5.
- 6. Negative myelomonocytic markers: CD13, CD33, CD11c, CD14, CD64, myeloperoxidase, chloroacetate esterase, butyrate esterase, lysozyme.
- 7. Negative NK cell-associated antigens: CD16, CD57, T-cell intracellular antigen 1, and perforin.
- 8. Negative progenitor cell markers: CD34, CD117
- 9. Negative lymphoid activation markers: CD25, CD30, CD71.
- 10. Other negative markers: CD10, CD21, CD35, CD36, S100, Epstein–Barr virus antigen.
- 11. Terminal deoxynucleotidyl transferase may be expressed in a primitive subset of BPDCN
- 12. T-cell receptor gene rearrangement analysis shows a germline pattern.

BDCA, Blood dendritic cell antigen; BPDCN, blastic plasmacytoid dendritic cell neoplasm; CD2AP, CD2-associated adaptor protein; CLA, cutaneous lymphocyte-associated antigen; TCL1, T-cell leukemia/lymphoma 1.

of LILRA4 and TCL1A transcripts in BPDCN cases that distinguished them from other acute leukemia cases (except for B-cell acute lymphoblastic leukemia).

In BPDCN cases, immunoglobulin heavy-chain gene rearrangement and T-cell receptor gene rearrangement usually show a germline pattern. T-cell receptor gamma or beta chain gene rearrangements, however, have been demonstrated in rare cases (2). The salient features for laboratory diagnosis of BPDCN are summarized in Table 6.15.2.

Clinical Manifestations

PBDCN is an aggressive neoplasm (2,6,8,11), usually encountered in elderly patients with a median age of about 65 years. The male/female ratio ranges from 2.5:1 to 3:1. The initial presentation usually involves the skin with solitary or multifocal lesions. It may appear from small bruise-like area to violaceous patches, nodules, and ulcerated masses. Extracutaneous involvement at presentation is mostly seen in draining lymph nodes, followed by spleen, liver and tonsil. Central nervous system is seldom involved at presentation but it is not unusual to be found in cases of relapse. Bone marrow or blood involvement may be existent at a low level in the early stage, but overt leukemia is usually

seen in advanced stage or relapse after therapy. B symptoms (weight loss, night sweats, and fever) are uncommon. Cytopenia, particularly thrombocytopenia, is a frequent clinical presentation. BPDCN has been reported to be associated with precedent, concurrent, or subsequent myelomonocytic leukemia (2,11). Whether this phenomenon represents direct transformation of BPDCN to myelomonocytic leukemia or vice versa or merely coexistence of two separate diseases has not been settled (31). However, nodules composed of PDCs have been frequently found in lymph nodes, skin, spleen, or bone marrow of patients with chronic myelomonocytic leukemia or other monocytic neoplasms, and some studies found that these PDC nodules have a clonal relationship with the leukemias (8,23).

Patients usually respond well to radiotherapy and/or chemotherapy initially, but relapse often occurs weeks or months later. The median survival period is approximately 12 to 14 months. Long-lasting remission is usually seen in patients who received an acute leukemia-type induction therapy followed by allogenic stem cell transplantation in first complete remission (2,32).

REFERENCES

- 1. Brody JP, Allen S, Schulman P, et al. Acute agranular CD4-positive natural killer cell leukemia. Comprehensive clinicopathologic studies including virologic and in vitro culture with inducing agents. *Cancer*. 1995;75:2474–2483.
- 2. Herling M, Jones D. CD4+/CD56+ hematodermic tumor. The features of an evolving entity and its relationship to dendritic cells. *Am J Clin Pathol*. 2007;127:687–700.
- 3. Chan JKC, Jaffe ES, Ralfkiaer E. Blastic NK-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:214–215.
- 4. Petrella T, Dalac S, Maynadie M, et al. CD4 + CD56 + cutaneous neoplasms: A distinct hematological entity? *Am J Surg Pathol*. 1999;23:137–146.
- 5. Petrella T, Comeau MR, Maynadie M, et al. “Agranular CD4+ CD56+ hematodermic neoplasm” (blastic NK-cell lymphoma) originates from a population of CD56+ precursor cells related to plasmacytoid monocytes. *Am J Surg Pathol*. 2002;26:852–862.
- 6. Patrella T, Bagot M, Willemze R, et al. Blastic NK-cell lymphomas (Agranular CD4+ CD56+ hematodermic neoplasms). *Am J Clin Pathol*. 2005;123:662–675.
- 7. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. 2005;105:3768–3785.
- 8. Jegalian AG, Facchetti F, Jaffe ES. Plasmacytoid dendritic cells. Physiologic roles and pathologic states. *Adv Anat Pathol*. 2009;16:392–404.
- 9. Marafioti T, Peterson JC, Ballabio E, et al. Novel markers of normal and neoplastic human plasmacytoid dendritic cells. *Blood*. 2008;111:3778–3792.
- 10. Comeau MR, Van der Vuurs de Vries AR, Maliszewski CR, et al. CD123 bright plasmacytoid predendritic cells: progenitor undergoing cell fate conversion? *J Immunol*. 2002;169:75–83.
- 11. Facchetti F, Jones DM, Petrella T. Blastic plasmacytoid dendritic cell neoplasm. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:343–349.

12. Garnache-Ottou F, Feuillard J, Saas P. Plasmacytoid dendritic cell leukaemia/lymphoma: towards a well defined entity? *Br J Haematol*. 2007;136:539–548.
13. Feuillard J, Jacob MC, Valensi F, et al. Clinical and biologic features of CD4+ CD56+ malignancies. *Blood*. 2002;99:1556–1563.
14. Bayerl MG, Rakozy CK, Mohamed AN, et al. Blastic natural killer cell lymphoma/leukemia. A report of seven cases. *Am J Clin Pathol*. 2002;117:41–50.
15. Pilichowska ME, Fleming MD, Pinkus JL, et al. CD4+ /CD56+ hematodermic neoplasm (Blastic natural killer cell lymphoma). Neoplastic cells express the immature dendritic cell marker BDCA-2 and produce interferon. *Am J Clin Pathol*. 2007;128:445–453.
16. Tsagarakis NJ, Kentrou NA, Papadimitriou KA, et al. Acute lymphoplasmacytoid dendritic cell (DC2) leukemia: Results from the Hellenic dendritic cell leukemia study group. *Leuk Res*. 2010;34:438–446.
17. Reineks EZ, Osei ES, Rosenberg A, et al. CD22 expression on blastic plasmacytoid dendritic cell neoplasms and reactivity of anti-CD22 antibodies to peripheral blood dendritic cells. *Cytometry Part B*. 2009;768:237–248.
18. Khoury JD, Medeiros LJ, Manning JT, et al. CD56+ TdT+ blastic natural killer cell tumor of the skin. A primitive systemic malignancy related to myelomonocytic leukemia. *Cancer*. 2002;94:2401–2408.
19. Jaye DL, Geigerman CM, Herling M, et al. Expression of the plasmacytoid dendritic cell marker BDCA2 supports a spectrum of maturation among CD4+ CD56+ hematodermic neoplasms. *Mod Pathol*. 2006;19:1555–1562.
20. Meyer N, Petrella T, Poszepczyrska-Guigne E, et al. CD4+ CD56+ blastic tumor cells express CD101 molecules. *J Invest Dermatol*. 2005;124:668–669.
21. Ferran M, Gallardo F, Ferrer AM, et al. Acute myeloid dendritic cell leukaemia with specific cutaneous involvement: a diagnostic challenge. *Br J Dermatol*. 2008;158:1129–1133.
22. Sun T, Pashael S, Jaffry I, et al. A hybrid form of myeloid/NK-cell acute leukemia and myeloid/NK-cell precursor acute leukemia. *Hum Pathol*. 2003;34:504–507.
23. Vermi W, Facchetti F, Rosati S, et al. Nodal and extranodal tumor-forming accumulation of plasmacytoid monocytes/interferon-producing cells associated with myeloid disorders. *Am J Surg Pathol*. 2004;28:585–595.
24. Del Giudice I, Matutes E, Morilla R, et al. The diagnostic value of CD123 in B-cell disorders with hairy cell or villous lymphocytes. *Haematologica*. 2004;89:303–308.
25. Herling M, Teitell MA, Shen RR, et al. TCL1 expression in plasmacytoid dendritic cells (DC2s) and the related CD4+ CD56+ blastic tumors of skin. *Blood*. 2003;101:5007–5009.
26. Petrella T, Meijer CJLM, Dalac S, et al. TCL1 and CLA expression in agranular CD4/CD56 hematodermic neoplasms (Blastic NK-cell lymphomas) and leukemia cutis. *Am J Clin Pathol*. 2004;122:307–313.
27. Garnache-Ottou F, Feuillard J, Ferrand C, et al. Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia. *Br J Haematol*. 2009;145:624–636.
28. Leroux D, Mugneret F, Callanan M, et al. CD4(+), CD56(+) DC2 acute leukemia is characterized by recurrent clonal chromosomal changes affecting 6 major targets: a study of 21 cases by the Groupe Francais de Cytogenetique Hematologique. *Blood*. 2002;99:4154–4159.
29. Dijkman R, van Doorn R, Szuhai K, et al. Gene-expression profiling and array-based CGH classify CD4+ CD56+ hematodermic neoplasm and cutaneous myelomonocytic leukemia as distinct disease entities. *Blood*. 2007;109:1720–1727.
30. Jardin F, Callanan M, Penther D, et al. Recurrent genomic aberrations combined with deletions of various tumor suppressor genes may deregulate the G1/S transition in CD4+ CD56+ haematodermic neoplasms and contribute to the aggressiveness of the disease. *Leukemia*. 2009;23:698–707.
31. Rasaiyaah J, Yong K, Katz DR, et al. Dendritic cells and myeloid leukaemias: plasticity and commitment in cell differentiation. *Br J Haematol*. 2007;138:28–290.
32. Dalle S, Beylot-Barry M, Bagot M, et al. Blastic plasmacytoid dendritic cell neoplasm: is transplantation the treatment of choice? *Br J Dermatol*. 2010;162:74–79.

CASE 16

Mixed Phenotype Acute Leukemia

CASE HISTORY

A 48-year-old man presented with high fever and severe back pain for several days. He started with rigors and chills with muscle spasms of the chest, moving around to the back. The pain then descended and localized in the back for the last 3 to 4 days. During the observation period, his platelets dropped rapidly from 208,000 to 30,000/u (mu)L in 3 weeks. He had epistaxis and oral mucosal bleeding. On admission, his total leukocyte count was 10,500/u (mu)L with 13% blasts in the peripheral blood. His hematocrit was 33% and hemoglobin 11.7 g/dL. Physical examination revealed no hepatosplenomegaly and no lymphadenopathy. A bone marrow biopsy demonstrated 94% blasts,

which had a high nuclear-to-cytoplasmic ratio and a thin rim of basophilic cytoplasm that contains many vacuoles (Figs. 6.16.1 and 6.16.2). The core biopsy showed almost total replacement of normal hematopoietic cells with undifferentiated blasts (Figs. 6.16.3 and 6.16.4).

FLOW CYTOMETRY

A CD45-negative population with CD10 58%, CD13 37%, CD19 97%, CD34 99%, CD79a 98%, myeloperoxidase (MPO) 89%, and terminal deoxynucleotidyl transferase (TdT) 97% (Fig. 6.16.5). Other markers including CD20, k, l, CD5, CD7, CD11b, CD14, CD33, CD64, and CD117 were all negative.

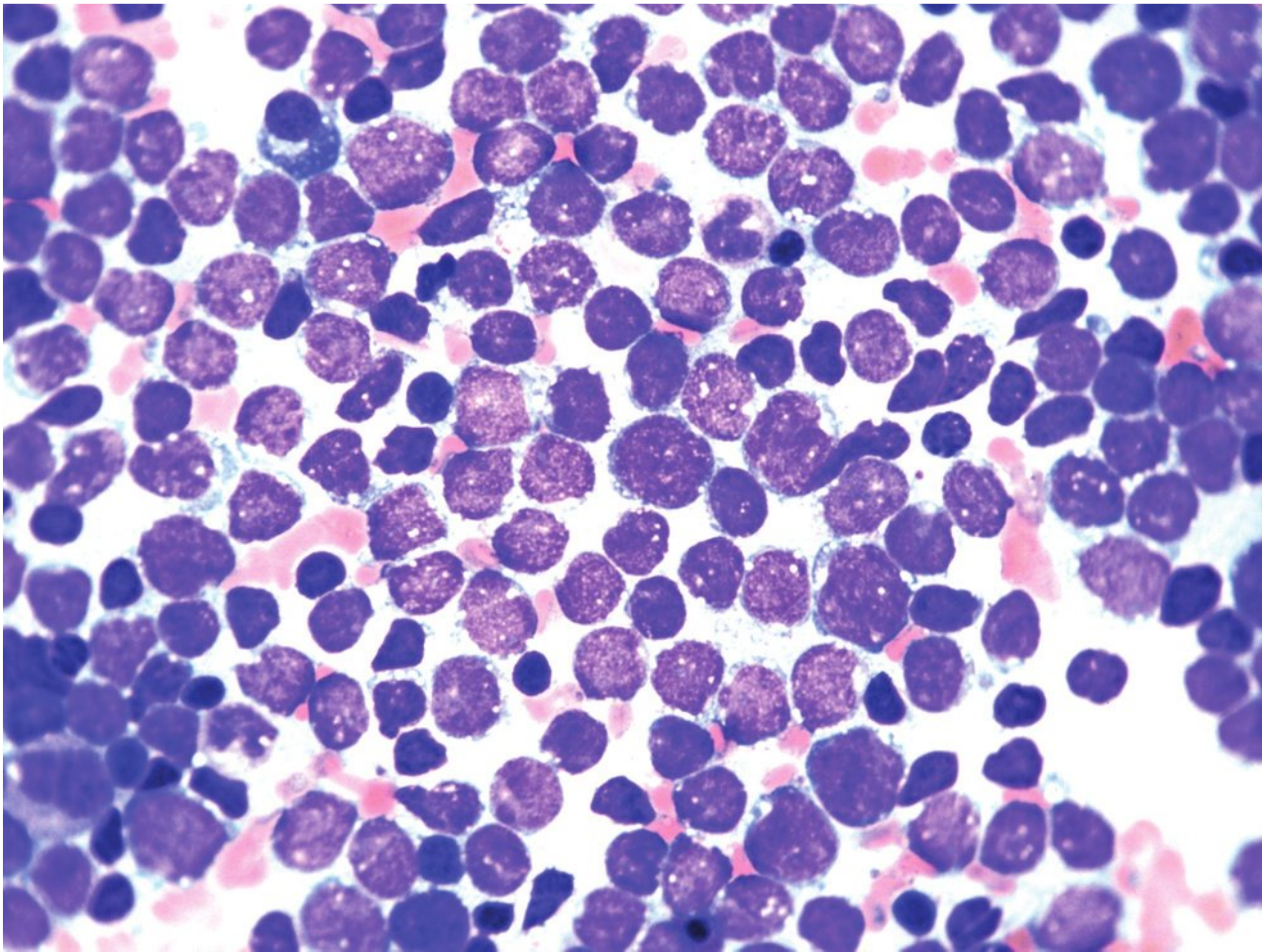


FIGURE 6.16.1 Bone marrow aspirate shows a pure blast population with high nuclear-to-cytoplasmic ratio. Wright-Giemsa, 60 × magnification.

MOLECULAR GENETICS

Fluorescence in situ hybridization (FISH) revealed BCR-ABL1 fusion gene.

Reverse transcriptase-polymerase chain reaction (RT-PCR) showed minor BCR-ABL fusion transcript (bcr/abl fusion protein 190 kDa).

FISH for MYC gene rearrangement was negative.

RT-PCR failed to identify Fms-like tyrosine kinase 3/internal tandem duplication (FLT3/ITD) mutation.

DISCUSSION

Most cases of acute leukemia can be classified into either myeloid or lymphoid leukemia by morphology and immunophenotyping. However, there are about 5% of acute

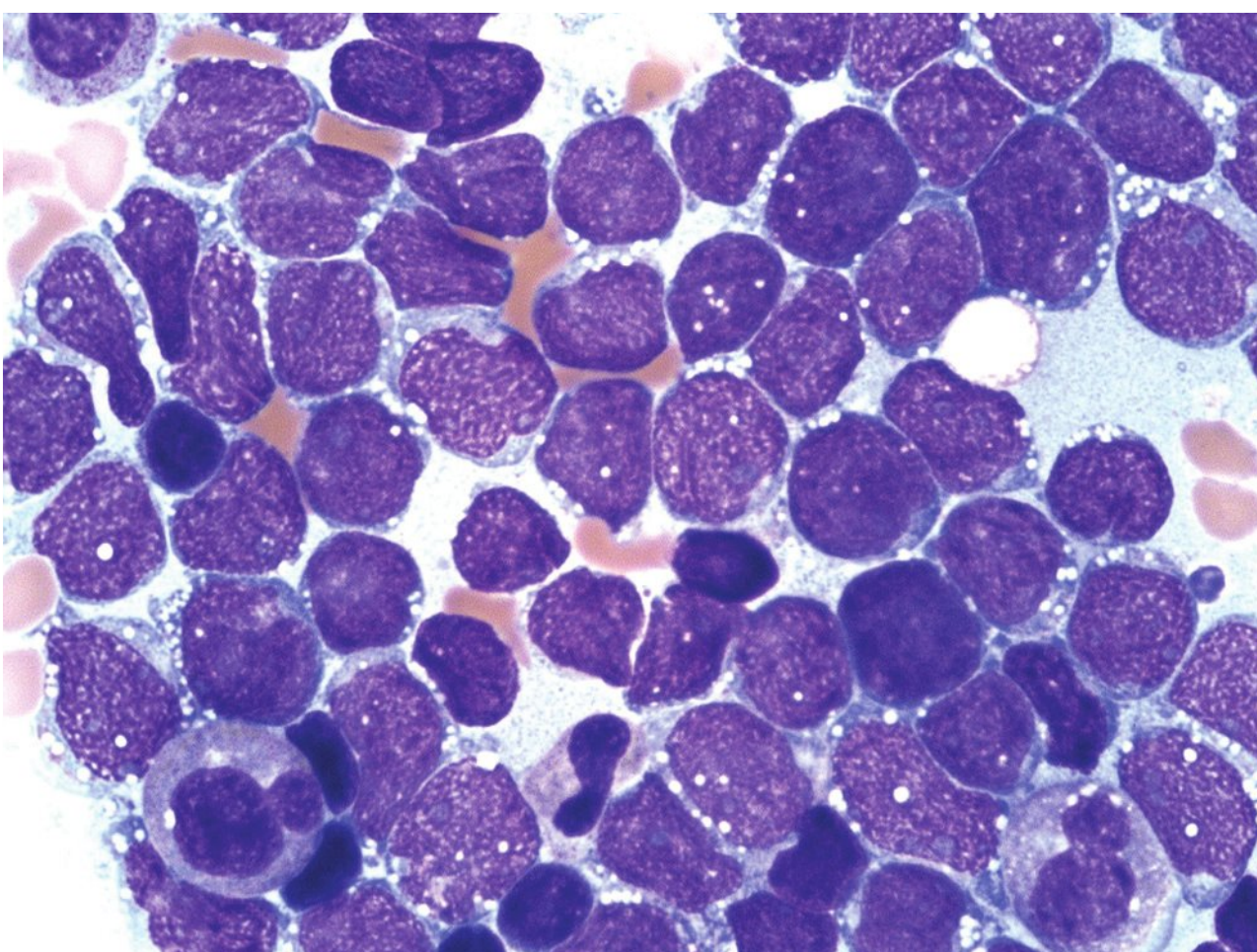


FIGURE 6.16.2 Higher magnification demonstrates many cytoplasmic vacuoles in the blasts. Wright-Giemsa, 100 × magnification.

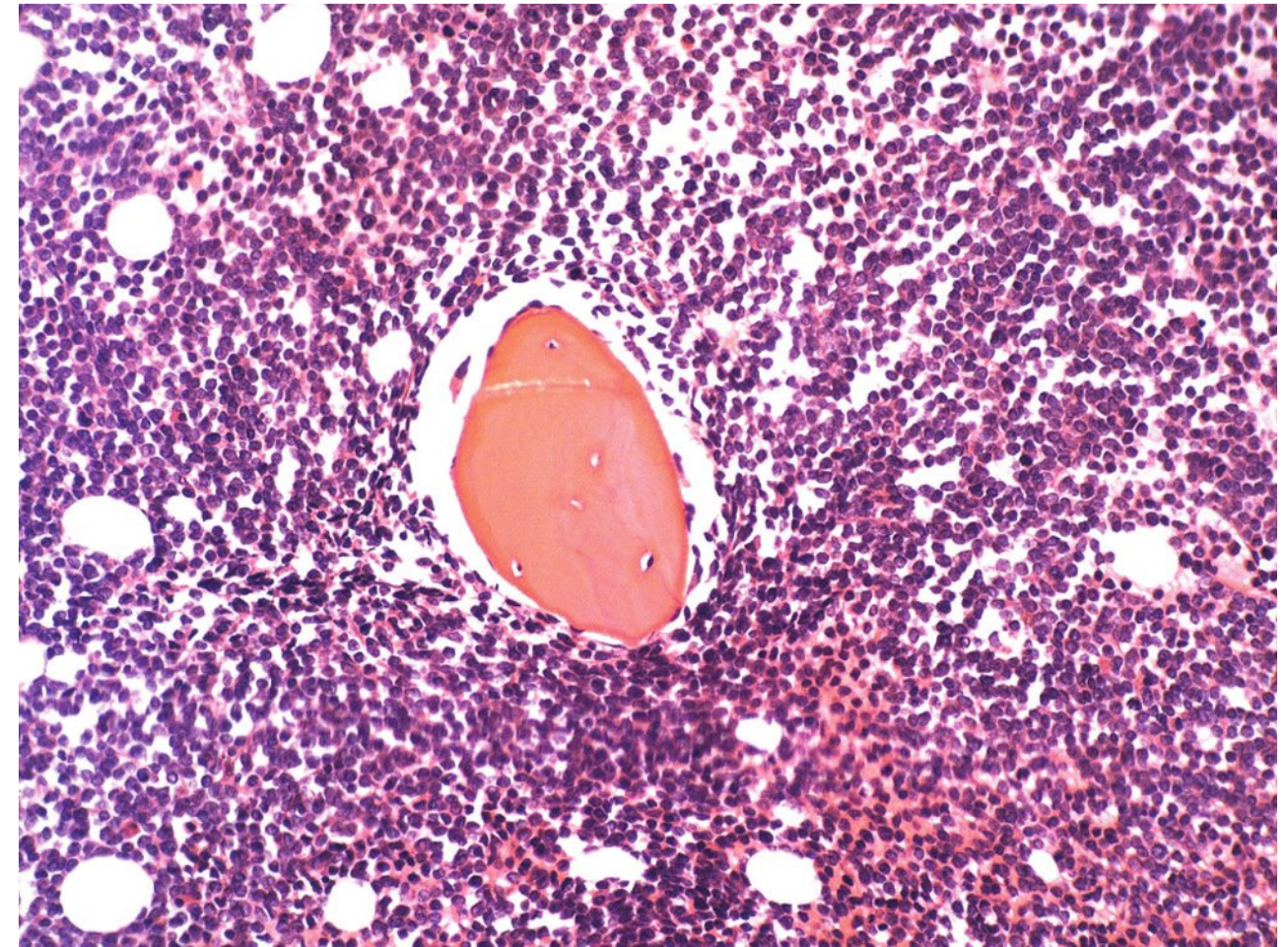


FIGURE 6.16.3 Bone marrow core biopsy reveals extensive leukemic infiltration, replacing normal hematopoietic cells. H&E, 20 × magnification.

leukemia cases that are difficult to identify even using the modern techniques (1). These cases are designated acute undifferentiated leukemia if both morphology and phenotyping are inconclusive (2). Cases consisting separate populations that have different morphologic and phenotypic features are termed acute bilineal or lineage leukemia (2). Cases showing only a single population that coexpresses two immunophenotypes are called biphenotypic acute leukemia (2). Biphenotypic acute leukemia accounts for 4% to 9% acute leukemia cases (3), while acute bilineal leukemia is about 1% to 2% (4). The World Health Organization (WHO) (2) classification combines the bilineal and biphenotypic leukemias into a single category, the mixed phenotype acute leukemias (MPALs).

For many years, the scoring system developed by the European Group for the Immunological Classification of Leukemia (EGIL) has been used to define biphenotypic

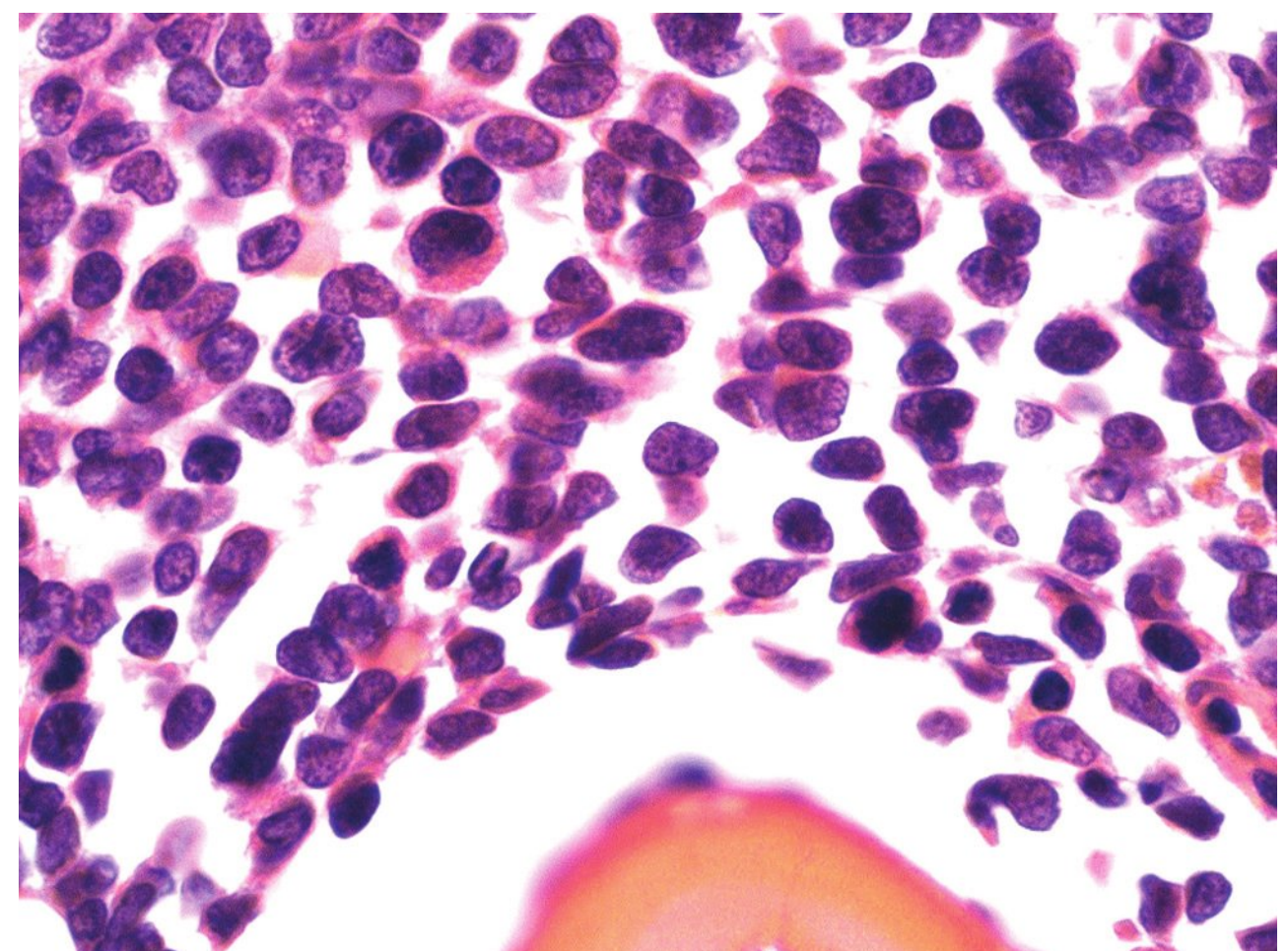


FIGURE 6.16.4 Higher magnification shows undifferentiated blast morphology. H&E, 100 × magnification.

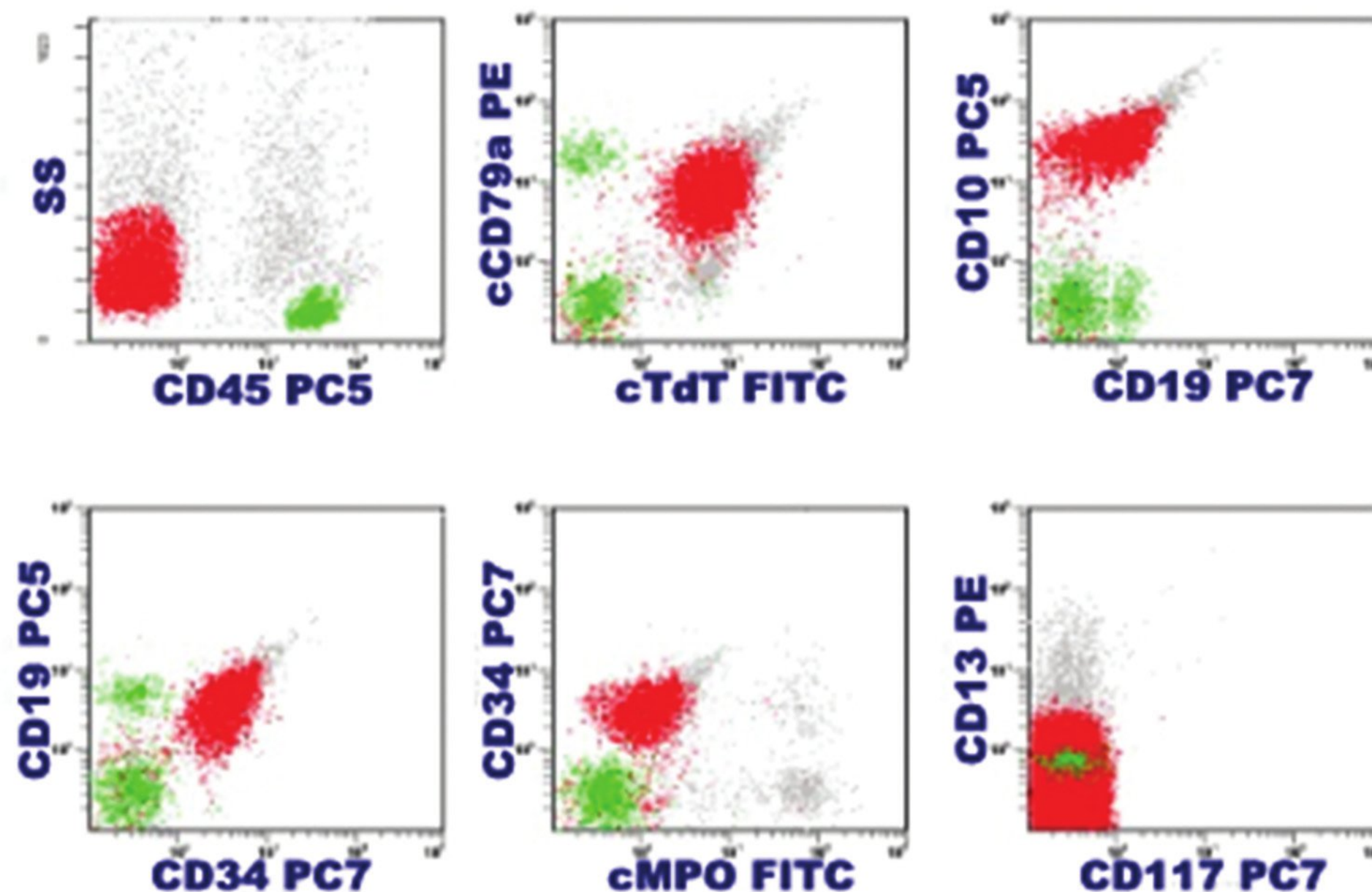


FIGURE 6.16.5 Flow cytometric histograms demonstrate a CD45-negative population that expresses CD79a, terminal deoxynucleotidyl transferase, CD10, CD19, CD34, MPO, CD13 but negative CD117.

leukemias (5–7). However, the 2008 WHO classification has adopted more restricted criteria for subclassification of MPAL (2), which will be discussed under immunophenotyping.

On the basis of immunophenotype, MPAL is further divided into B/myeloid, T/myeloid, and rare type (2). The rare type includes mixed phenotype of T cell and B cell or trilineage phenotype (T cell, B cell, and myeloid cell). Mixed phenotypes involving lymphoid/megakaryoblast or lymphoid/erythroblast have not been reported. Two karyotypes are singled out as distinct entities because of their high frequency. They are $t(9;22)(q34;q11.2)$ or BCR-ABL1 and $t(v;11q23)$ or MLL (mixed lineage leukemia gene) rearrangement. Three karyotype-defined acute myeloid leukemia (AML) entities, $t(8;21)$, $t(15;17)$, and $inv(16)$, are excluded from MPAL, even when multiple lymphoid markers are detected in these cases (2). Acute leukemia with FGFR1 mutation is also excluded (2). If lymphoid phenotype is expressed in chronic myelogenous leukemia in blast crises, or in myelodysplastic syndrome-related AML, and therapy-related AML, the diagnosis of these entities will remain the same with a secondary notation that they have a mixed phenotype (2).

Morphology

As mentioned before, bilineal leukemia shows two blast populations; one may resemble lymphoblasts and the other myeloblasts (2). In MPAL with MLL rearrangement or $t(v;11q23)$, the mixed population is usually composed of lymphoblasts and monoblasts. In MPAL with BCR-ABL1 or $t(9;22)$, it should be distinguished from chronic myelogenous leukemia with lymphoblast crisis. There are no

distinguishing morphologic features between B/myeloid and T/myeloid cases. If there is only a single blast population, the blasts show no distinguishing features or are undifferentiated (2). When myelodysplastic changes are present, myelodysplastic syndrome-related AML should be excluded. If the patient has a history of receiving topoisomerase II inhibitors, therapy-related myeloid leukemia should be ruled out. Patients with topoisomerase II inhibitor therapy also show MLL rearrangement frequently (8).

Immunophenotype

The scoring system developed by the EGIL includes many markers (Table 6.16.1) (5–7). An immunophenotype is established when a score is >2 . A marker is considered positive when they are expressed in $>20\%$ of blasts. For MPO and TdT, 10% is sometimes used as the cutoff.

The WHO system designates MPO, CD3, and CD19 as the specific markers for myeloid, B-cell, and T-cell lineages, respectively (2). For myeloid lineage, only MPO is accepted for its identification. Other myeloid markers, such as CD13, CD33, and CD117, are deemed nonspecific for lineage identity. For monocytic lineage, at least two of the following markers should be positive for its identification. These markers include nonspecific esterase, CD11c, CD14, CD64, and lysozyme. CD3, no matter whether it is cytoplasmic or surface in location, is the only acceptable marker for T-cell identity. It is cautioned that immunohistochemistry using polyclonal CD3 antibody may detect CD3 zeta chain, which is not T-cell specific. Identification of B-cell lineage requires more than one marker. If CD19 staining is strong, coexpression with CD79a, CD10, or cytoplasmic CD22 is required. If CD19 staining is weak, coexpression with two

TABLE 6.16.1			
Scoring System for Markers Proposed by the EGIL			
Score	B-lymphoid	T-lymphoid	Myeloid
2	cCD79a*	CD3/cCD3	MPO
	cIgM	Anti-TCR	
	cCD22		
1	CD19	CD2	CD117
	CD20	CD5	CD13
	CD10	CD8	CD33
		CD10	CD65
0.5	TdT	TdT	CD14
	CD24	CD7	CD15
		CD1a	CD64

*CD79a may also be expressed in some cases of precursor T-lymphoblastic leukemia/lymphoma.
 C, cytoplasmic; MPO, myeloperoxidase; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase; Ig, immunoglobulin.

of the above three markers is required. Flow cytometry is the preferred method for detection of these markers. Immunohistochemistry and cytochemistry are also acceptable for detection of certain markers, such as MPO and nonspecific esterase. One recent study has recommended the use of electron microscopy for identification of MPO and concluded that this technique is more sensitive than cytochemistry and flow cytometry for the detection of MPO (9).

In MPAL with MLL rearrangements in pediatric patients, the most predictive immunophenotype is CD10[−], CD24[−], CDw65⁺, CD15⁺, NG2⁺ (chondroitin sulfate proteoglycan neuron-glia antigen 2) (10–12). NG2 is 100% specific for the prediction of MLL gene rearrangement. In addition, these cases usually show negative CD20, CD79a, CD13, and CD33 but high levels of CD43 and CD44 (10,11). This immunophenotype is seen mainly in pro-B and CD10-negative pre-B lymphoblastic leukemias with myeloid components (11).

In a study of nine cases of B/myeloid MPAL, all cases were positive for CD19 and CD22, but only six of eight cases tested were positive for CD20 (4). In these nine cases, only seven cases showed positive MPO; thus, two cases should be excluded by the new WHO criteria. Other myeloid markers, including CD13, CD33, CD15, CD11b, and CD117, were expressed variably. Three of the seven cases also demonstrated monocyte markers, CD14 and CD64. In a study of 10 cases of T/myeloid MPAL, all cases expressed surface CD3 and CD7 (4). MPO was expressed in 7 of 8 cases studied, CD13 was shown in all cases and CD33 in 9 of 10 cases. Most studies showed that B/myeloid cases are more common than T/myeloid cases with an average ratio about 2:1 (9). Mixed T-cell and B-cell leukemia is rare. It is cautioned that CD79a and CD10 can be demonstrated in T-cell leukemia and if CD3 is expressed, the case should not be classified as T/B MPAL (2). There have been no cases with

coexpression of lymphoid and erythroid or megakaryocytic markers reported.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is preferred to immunohistochemistry for immunophenotyping of MPAL because the former can clearly quantify the three essential markers, MPO, CD3 and CD19. There is no CD19 antibody for immunohistochemical staining. Most importantly, flow cytometry may readily demonstrate multiple markers on the same cells and reveals the biphenotypic or triphenotypic nature of the case, as illustrated in the current patient.

Molecular Genetics

The major cytogenetic aberrations in MPAL are t(9;22)(q34;q11.2);BCR-ABL1 and t(v;11q23);MLL rearranged, which are included as distinct subtypes of MPAL by WHO. The former is the most common recurrent genetic abnormality in general with a frequency of 28% to 35% (9), but it only accounts for <1% of acute leukemias (2). MLL rearrangements, on the other hand, are more commonly seen in pediatric cases, particularly in infancy, but it accounts for 10% to 32% in general (9). However, these abnormal karyotypes are mainly seen in B/myeloid MPAL (4). T/myeloid cases may show frequent cytogenetic aberrations but these are generally nonrecurring (4). Other rare karyotypic aberrations found in MPAL include 5q[−], 6q[−], 12p[−], and 12p11.2 abnormalities (4,9).

MLL has more than 30 partner genes, including MLL-AF4 or t(4;11), MLL-AF10 or t(10;11), MLL-AP9 or t(9;11), and MLL-ENL or t(11;19) (13). MLL rearrangement can be demonstrated by either RT-PCR or FISH. Using a dual-color MLL split apart probe in FISH may detect virtually all MLL gene involved in translocation, and translocation-specific FISH probes can be used to identify the specific partner gene (10). MLL gene normally functions as a transcription regulator of the homeodomain (HOX) genes. When MLL is translocated, the fusion transcripts produce fusion proteins that disrupt the ability of wild-type MLL to regulate class I HOX gene expression, leading to leukemogenesis (13). Among the Hox genes, Hexa9 shows a relatively high expression in MPAL cases (14). Similar to HOX gene, NG2 gene also exhibits a high expression in cases with MLL rearrangement (13). With microarray studies, FLAT3 gene is also shown to be overexpressed in MLL rearranged cases and is a potential therapeutic target (8). FLT3 is a receptor tyrosine kinase and should be susceptible to tyrosine kinase inhibitors (15).

A gene expression profiling study of acute myeloid and lymphoid leukemias with and without MLL rearrangements identified an MLL translocation-specific signature and a phenotype-related signature (13). The former includes eight genes (MEIS1, ZEB2, SRGAP2P1, TMEM30A, AK2, TMED2, HIPK3, and FAM62B) that identify cases with MLL translocation, while the latter includes six genes (PAX5, CD72, CSRP2, LOC100130458, EBF1, and TCL1A) that help distinguish acute lymphoblastic leukemia (ALL) from AML. Gene expression profiling may also predict ALLs with MLL, BCR-ABL1, and T-ALL (16).

TABLE 6.16.2

Salient Features for Laboratory Diagnosis of MPAL

1. One to three blast populations expressing phenotypes of two or three cell lineages
2. Major subgroups include MPAL with t(9;22) (q34;q11.2);BCR-ABL1, MPAL with t(v;11q23);MLL rearranged, MPAL B/myeloid, NOS, MPAL T/myeloid, NOS, and MPAL, NOS—rare type (e.g., mixed T cell/B cell, trilineage)
3. Myeloid lineage is defined by myeloperoxidase or in case of monocytic differentiation, defined by two of the monocyte markers (nonspecific esterase, CD11c, CD14, CD64, lysozyme)
4. T-cell lineage is defined by cytoplasmic or surface CD3.
5. B-cell lineage is defined by multiple B-cell markers.
 - a. If CD19 is strong, strong coexpression of CD79a, cytoplasmic CD22, or CD10 is required.
 - b. If CD19 is weak, strong coexpression of two of the above B-cell markers are required.

Immunoglobulin heavy chain (IgH) gene and T-cell receptor (TCR) gene rearrangements have been reported only in a few cases of biphenotypic acute leukemias. One study showed that four of nine cases had rearrangement of both IgH and TCR genes (9). A few other studies also emphasized the dual rearrangement as a valuable tool for follow-up of biphenotypic acute leukemias. The salient features for laboratory diagnosis of MPAL are listed in Table 6.16.2.

The current case showed undifferentiated leukemic features in the bone marrow cord biopsy, but the marrow aspirate revealed large blasts with vacuolated cytoplasm mimicking Burkitt leukemia. The immunophenotype showed coexpression of both myeloid (MPO, CD13) and B-cell (CD19, CD79a) markers that fulfill the diagnostic criteria of both WHO and EGIL. Karyotyping was unsuccessful due to the absence of mitosis in the cell culture. Therefore, several molecular genetic tests were performed. MYC rearrangement was done because of suspicion for Burkitt lymphoma. BCR-ABL1 was done because of the high frequency of this aberration in MPAL. FLT3 was tested because of its high incidence in acute leukemia and its potential for therapy with inhibitors. The detection of minor BCR-ABL fusion is consistent with BCR-ABL1-positive ALL and not chronic myelogenous leukemia. The patient was treated with imatinib mesylate and chemotherapy and he was still alive 3 years after initial diagnosis.

Clinical Manifestations

Cases of MPAL have no specific clinical features to distinguish from other acute leukemias. MPAL cases with t(9;22) may behave like Ph+ ALL with high leukocyte counts and worst prognosis than other MPAL subgroups (2). MPAL cases with MLL rearrangement also show high white blood

cell counts and also carry a poor prognosis (2). B/myeloid and T/myeloid cases fare equally badly with no difference in the survival between these two groups of patients (4,17). However, the immunophenotype of each case may change. For instance, bilineal leukemia (two-blast populations) may change to biphenotypic leukemia (single-blast population). MPAL may also switch to pure ALL or AML after treatment (2,4).

There are no standard therapeutic regimens for MPAL patients. They have been treated with ALL, AML, or combined ALL/AML regimens. One study found that ALL regimens were better than AML regimens with the complete remission rates of 78% and 57%, respectively (17). When BCR-ABL1 or FLT3 is identified, tyrosine kinase inhibitors have been tried for therapeutic purposes (15). Stem cell transplantation has also been used (4).

REFERENCES

1. Matutes E, Morilla R, Farahat N, et al. Definition of acute biphenotypic leukemia. *Haematologica*. 1997;82:64–66.
2. Borowitz MJ, Bene MC, Harris NL, et al. Acute leukaemias of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:150–155.
3. Kroft SH, Karandikar NJ. Flow cytometric analysis of acute leukemias, myelodysplastic syndromes, and myeloproliferative disorders. In: Carey JL, McCoy JP, Keren DE, eds. *Flow Cytometry in Clinical Diagnosis*. 4th ed. Chicago, IL: ASCP Press; 2007:167–214.
4. Weir EG, Ansari-Lari MA, Batista DAS, et al. Acute bilineal leukemia: a rare disease with poor outcome. *Leukemia*. 2007;21:2264–2270.
5. Matutes E, Catovsky D. The value of scoring systems for the diagnosis of biphenotypic leukemia and mature B-cell disorders. *Leuk Lymphoma*. 1994;13(suppl 1):11–14.
6. Bene MC, Bernier M, Casasnovas RO, et al. The reliability and specificity of c-kit for the diagnosis of acute myeloid leukemias and undifferentiated leukemias. *Blood*. 1998;92:596–599.
7. European Group for the Immunological Classification of Leukaemias. The Value of c-kit in the diagnosis of biphenotypic acute leukemia. *Leukemia*. 1998;12:2038.
8. Chowdhury T, Brady HJM. Insights from clinical studies into the role of the MLL gene in infant and childhood leukemia. *Blood Cell Mol Dis*. 2008;40:192–199.
9. Owaidah TM, Beihany AA, Iqbal MA, et al. Cytogenetics, molecular and ultrastructural characteristics of biphenotypic acute leukemia identified by the EGIL Scoring system. *Leukemia*. 2006;20:620–626.
10. Attarbaschi A, Mann G, König M, et al. Mixed lineage leukemia-Rearranged childhood pro-B and CD10-negative pre-B acute lymphoblastic leukemia constitute a distinct clinical entity. *Clin Cancer Res*. 2006;12:2988–2994.
11. Borkhardt A, Wuchter C, Viehmann S, et al. Infant acute lymphoblastic leukemia—combined cytogenetic, immunophenotypical and molecular analysis of 77 cases. *Leukemia*. 2002;16:1685–1690.
12. Schwartz S, Rieder H, Schlager B, et al. Expression of the human homologue of rat NG2 in adult acute lymphoblastic leukemia: close association with MLL rearrangement and a CD10(-)/CD24(-)/CD65a(+)/CD15(+) B-cell phenotype. *Leukemia*. 2003;17:1589–1595.

13. Zangrando A, Dell'Orto MC, te Kronnie G, et al. MLL rearrangements in pediatric acute lymphoblastic and myeloblastic leukemias: MLL specific and lineage specific signatures. *BMC Med Genomics*. 2009;2:36–47.
14. Ernst P, Wang J, Korsmeyer SJ. The role of MLL in hematopoiesis and leukemia. *Curr Opin Hematol*. 2002;9:282–287.
15. Armstrong SA, Kung AL, Mabon ME. Inhibition of FLT3 in MLL. Validation of a therapeutic target identified by gene expression based classification. *Cancer Cells*. 2003;3:173–183.
16. Kohlmann A, Schoch C, Schnittger S, et al. Pediatric acute lymphoblastic leukemia (ALL) gene expression signatures classify an independent cohort of adult ALL patients. *Leukemia*. 2004;18:63–71.
17. Aribi A, Bueso-Ramos C, Estey E, et al. Biphenotypic acute leukaemia: a case series. *Br J Haematol*. 2007;138:213–216.

CASE 17

B-Lymphoblastic Leukemia/Lymphoma

CASE HISTORY

A 20-year-old man was found to have leukocytosis during a preoperative evaluation before a knee operation. Further hematologic workup showed that his total leukocyte count was 55,000/mL with 54% lymphocytes, 33% blasts, 11% neutrophils, and 2% monocytes. The hematocrit was 45% and platelet count 331,000/mL. The only clinical symptom that the patient had at that time was a persistent sore throat.

The patient was initially refractory to chemotherapy, but he was finally in remission after high-dose chemotherapy. However, his leukocyte count gradually dropped to 100/mL. His hematocrit dropped to 23.6% and platelets to 30,000/mL. He developed a neutropenic fever with a temperature of 101.2°F. His infection was finally under control, and he received a bone marrow transplant from a matched, unrelated donor.

The patient was doing well for 10 months without any clinical symptoms, but his routine checkup showed multiple blasts at the end of the 10th month. He was again treated with chemotherapy, which achieved a complete remission. However, he developed pulmonary aspergillosis with subsequent spreading to the brain. He had several episodes of left-sided seizures with residual left lower-extremity weakness. Despite multiple antibiotic treatments, the patient continued to have spiking fever, and became lethargic and confused. The patient finally died 17 months after the initial diagnosis of acute lymphoblastic leukemia (ALL).

FLOW CYTOMETRIC FINDINGS

Bone marrow aspiration: CD7 0%, CD10 94%, CD10/CD19 84%, CD14 1%, CD13-CD33 53%, CD34 97%, k 0%, l 0%, terminal deoxynucleotidyl transferase (TdT) 85% (Fig. 6.17.1).

CYTOCHEMICAL STAINS

The blasts were negative for myeloperoxidase (MPO), α-naphthyl butyrate esterase, and chloroacetate esterase but were positive for periodic acid-Schiff (PAS).

DISCUSSION

Precursor B-lymphoblastic leukemia/lymphoma includes ALL and lymphoblastic lymphoma (LBL) of B-cell origin. ALL is a leukemia with proliferation of lymphoblasts involving both the bone marrow (Fig. 6.17.2) and the peripheral blood. The cutoff point for clinical diagnosis of ALL is 25% blasts in the bone marrow (1). A case is diagnosed as ALL when ≥25% of lymphoblasts are present in the marrow. If bone marrow shows <25% of lymphoblasts in a case with lymph node or other soft tissue involvement, it is designated LBL with bone marrow involvement. There is a need to establish an arbitrary threshold to separate ALL from LBL because a leukemic phase may be present in LBL. In contrast, lymphoblasts may be absent in the peripheral blood in occasional ALL cases (aleukemic leukemia). In fact, about one third of ALL patients have a total white cell count of <5,000/μL.

Morphology and Cytochemistry

The morphology of leukemic lymphoblasts varies between adults and children. The most popular morphologic classification is the French-American-British (FAB) system, which divides ALL into L1, L2, and L3 (Table 6.17.1) (2,3). The leukemic cells in L1 are uniformly small with scanty cytoplasm (Fig. 6.17.3). Their nuclei are regular in shape, with inconspicuous nucleoli. This form is usually seen in pediatric cases. The neoplastic cells in L2 are generally large, but their size is variable, as is the cytoplasm (Fig. 6.17.4). Their nuclei also vary in shape, with

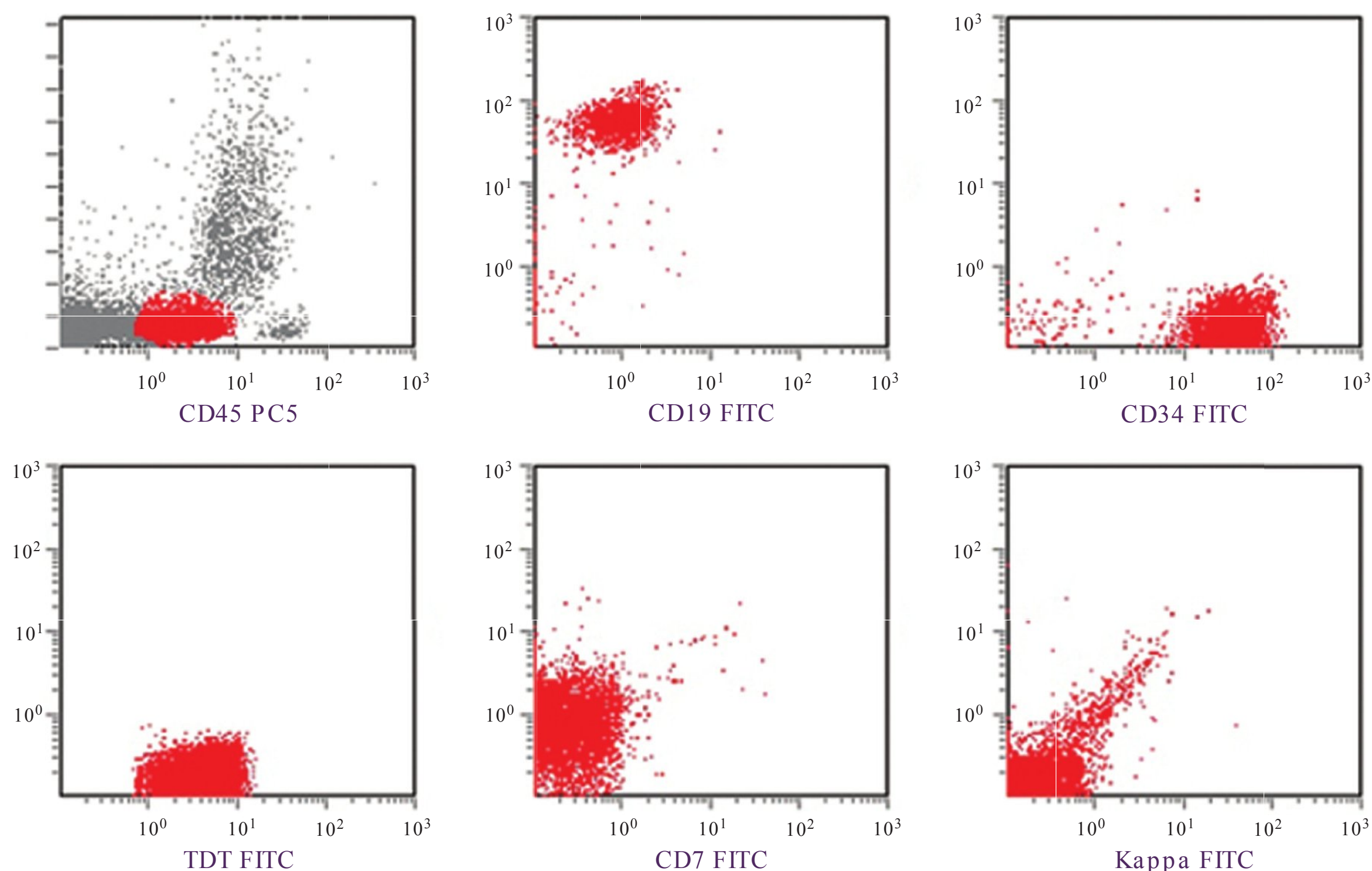


FIGURE 6.17.1 Flow cytometric histograms show dual staining of CD10 and CD19, positive CD34 and TdT, partial positive CD13.33, but negative CD7, kappa, and lambda. SS, side scatter; PC5, phycoerythrin–cyanin 5; RD1, rhodamine; FITC, fluorescein isothiocyanate; TdT, terminal deoxynucleotidyl transferase; PE, phycoerythrin.

prominent nucleoli. This form is more frequently seen in adults than in children. The cells in L3 are uniformly large, with moderate amounts of deep basophilic cytoplasm, which contains many vacuoles. The nuclei are round and regular with prominent nucleoli. This form is rare in comparison with L1 and L2 and is more frequently

seen in adults. It can also be the leukemic form of Burkitt lymphoma. However, the 2008 WHO classification discourages the use of L3 ALL to describe Burkitt lymphoma/leukemia (4).

Nevertheless, not all cases of ALL are easily assigned to the L1 and L2 subgroups, so reproducibility among observers is not high (5). In addition, immunophenotypes and cytogenetic abnormalities play an important role in predicting the prognosis, but these parameters do not correlate well with the L1 and L2 classifications. Therefore, L1 and L2 have been combined by the World Health Organization (WHO) into one group and the FAB nomenclature is no longer used (5,6). ALL must be distinguished from acute myeloid leukemia (AML). Their distinction is based on morphology, cytochemistry, immunophenotyping, and genotyping (Table 6.17.2).

Cytochemically, lymphoblasts are only positive for PAS (7). The typical staining pattern for lymphoblasts is called the block (coarse-granular) pattern, but this pattern is not always present. In fact, some ALL cases can be PAS negative. In contrast, cases of AML may occasionally show a positive reaction to PAS. Therefore, the PAS reaction is not specific. On the other hand, MPO, chloroacetate esterase, and alpha-naphthyl butyrate esterase are relatively specific for AML, so negative reactions to these cytochemical stains are helpful in excluding AML and are thus useful in establishing the diagnosis of ALL.

Another entity that should be distinguished is the hematogone. Hematogones are normal B-cell precursors that can be demonstrated in pediatric bone marrow or in

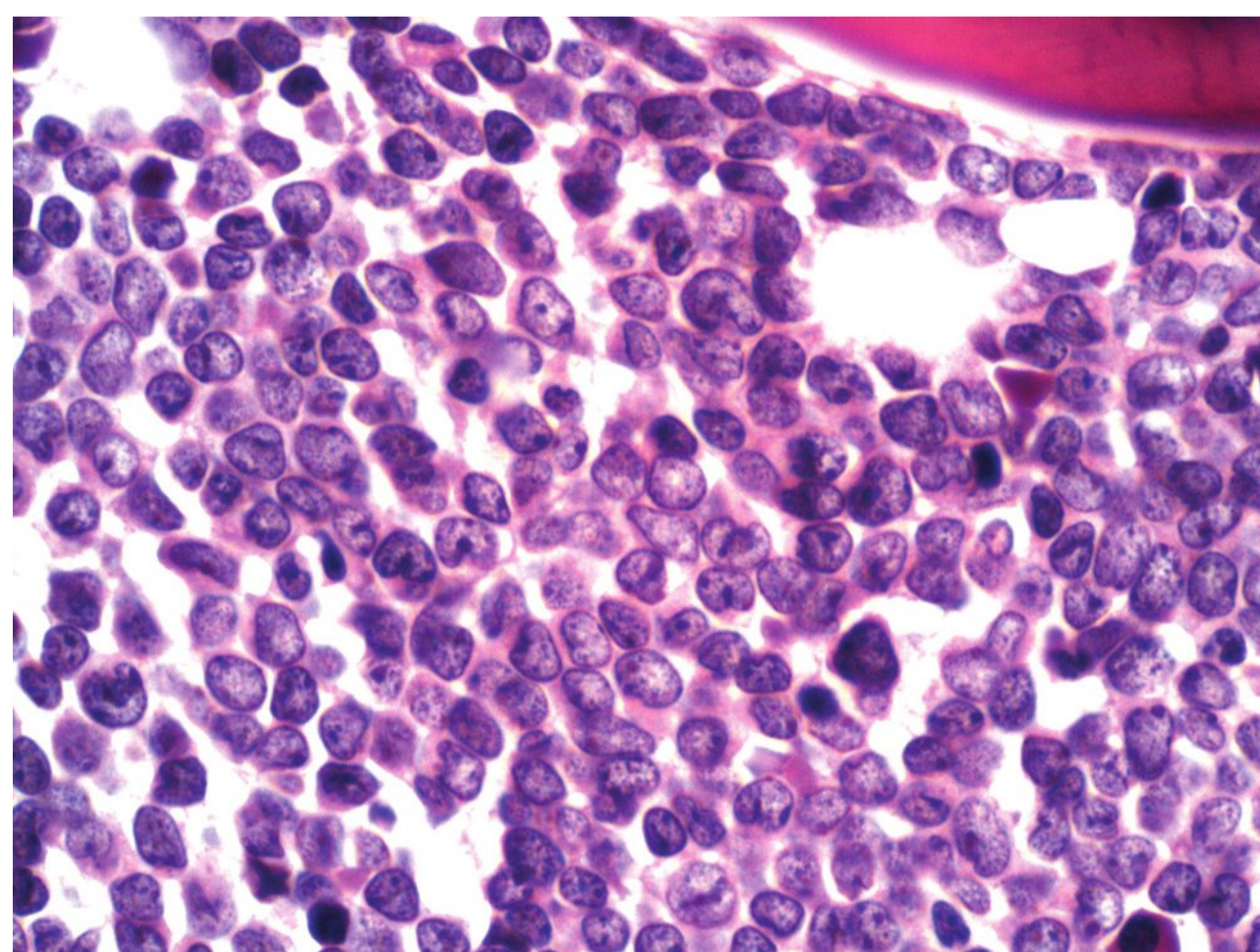


FIGURE 6.17.2 Bone marrow biopsy of a case of B-cell ALL shows total replacement of normal hematopoietic cells with lymphoblasts that shows convoluted or folded nuclei. Hematoxylin and eosin, 100× magnification.

TABLE 6.17.1			
FAB Classification for ALL			
	L1	L2	L3
Size of blasts	Small, uniform	Large, variable	Medium to large, uniform
Amount of cytoplasm	Scanty	Variable	Moderate
Cytoplasmic basophilia	Moderate	Variable	Intense
Cytoplasmic vacuoles	Variable	Variable	Prominent
Nucleus	Regular, occasional clefting, homogeneous chromatin	Irregular, clefting common, heterogeneous chromatin	Regular, noncleaved, homogeneous, finely stippled chromatin
Nucleolus	0–1, inconspicuous	≥1, prominent	2–5, prominent
N/C ratio	High	Low	Low

FAB, French-American-British; N/C ratio, nuclear/cytoplasmic ratio.

bone marrow regenerating after chemotherapy or transplantation. It is, therefore, most important to differentiate hematogones from lymphoblasts, particularly in pediatric ALL cases after chemotherapy. Hematogones are small-to-medium sized with a high nuclear cytoplasmic ratio and can mimic small mature lymphocytes or L1 lymphoblasts. Their major morphologic differences from lymphoblasts are the homogeneous nuclear chromatin pattern and the absence of nucleoli (6). In some cases, however, indistinct nucleoli can be present. A low percentage (0.01% to 1.3%) of hematogones has been detected in the peripheral blood of patients without ALL (8).

In lymph nodes and other solid organs, LBL is characterized by a diffuse infiltration and, less commonly

a paracortical pattern (Fig. 6.17.5) (4,6). In soft tissue, a single-file infiltration pattern is common (Fig. 6.17.6).

Immunophenotype

In the Foon and Todd (9) immunologic classification, antibodies against HLA-DR, CD19, CD10, CD20, Cm and surface IgM are used to subdivide B-cell ALL into six subgroups. However, some of the subgroups may not be relevant in terms of prognosis and treatment. Therefore, the new immunologic classification includes only three subgroups: B-precursor ALL, pre-B ALL, and B-ALL (10). Some authors use the terms early pre-B, pre-B, and B-ALL to define the same classification (11). Others omit the pre-B stage (1) or add a transitional pre-B subgroup in between pre-B and B-ALL (12). The 2008 WHO

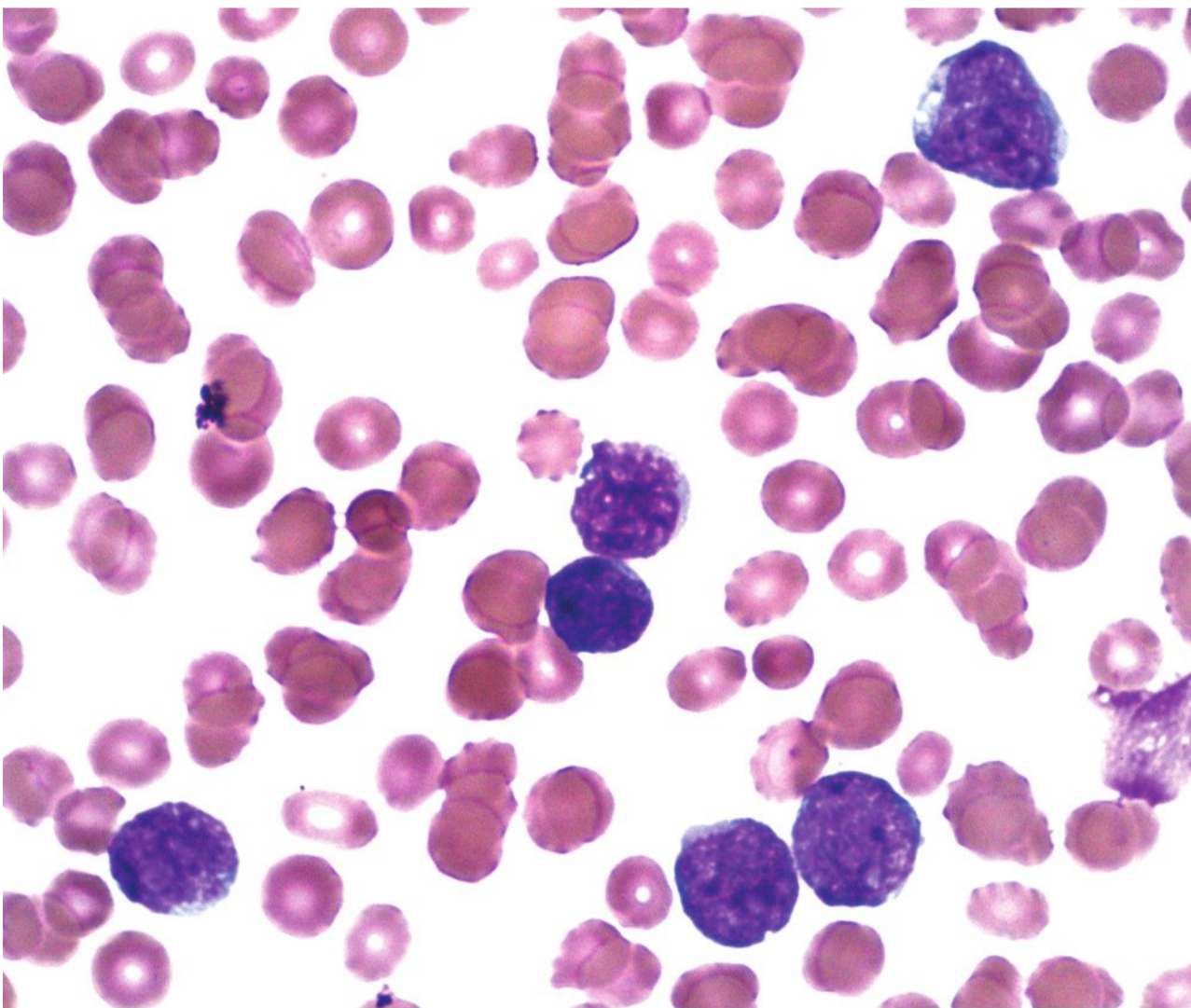


FIGURE 6.17.3 Peripheral blood smear of ALL case shows small, uniform blasts with scant cytoplasm and inconspicuous nucleoli (L1 morphology). Wright–Giemsa, 100× magnification.

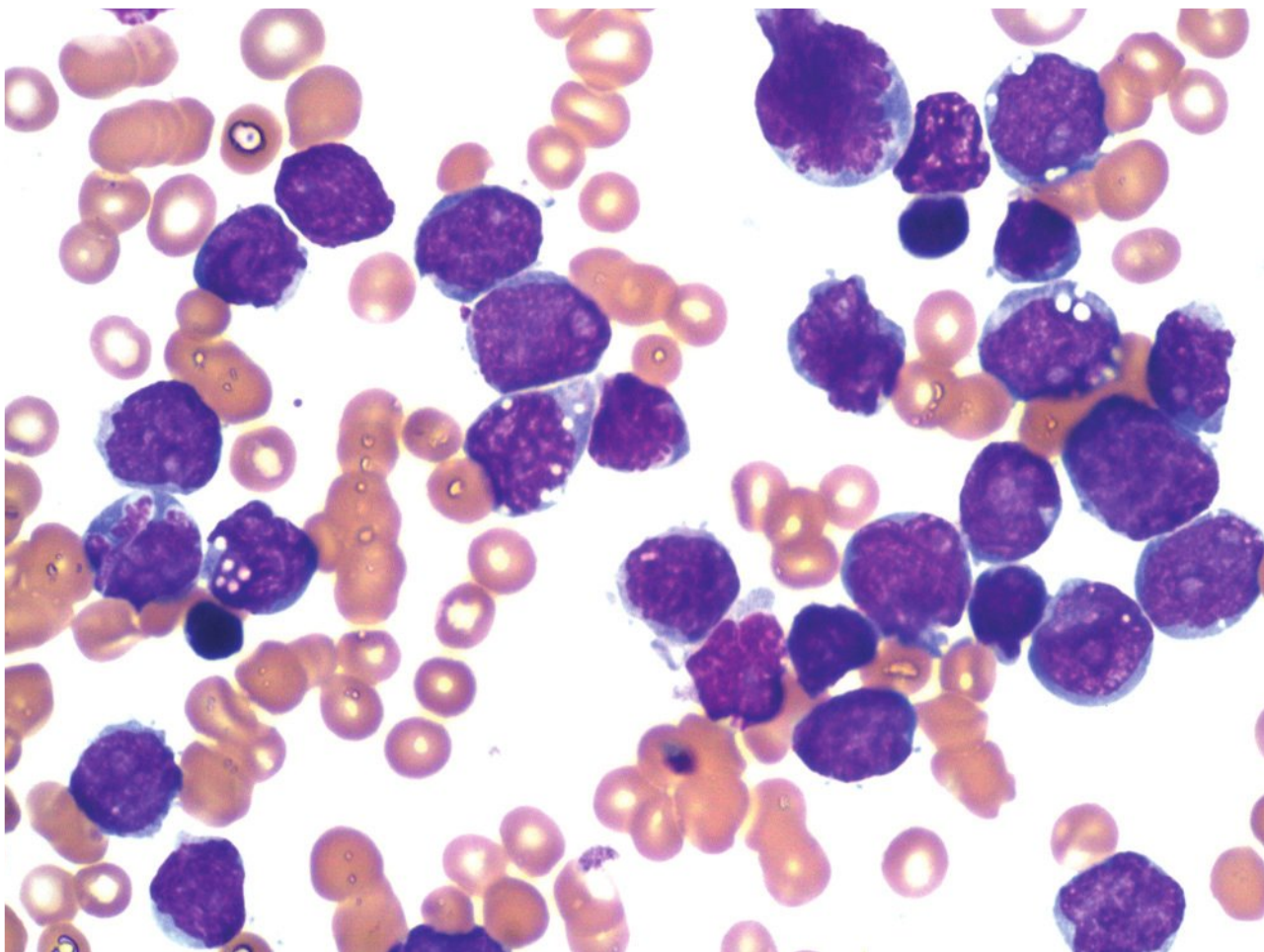


FIGURE 6.17.4 Bone marrow aspirate of ALL case shows lymphoblasts with variable sizes, scant cytoplasm with vacuolation in a few cells, and convoluted nucleus (L2 morphology). Wright–Giemsa, 100× magnification.

TABLE 6.17.2

Differentiating Features between Acute Lymphoblastic and Acute Myeloblastic Leukemias

	Lymphoblastic	Myeloblastic
Size of blasts	Variable, depending on subtype	Usually large and uniform
Cytoplasm	Scant	Moderate amount
Cytoplasmic granules	Absent	Frequently present
Auer rods	Absent	Seen in about 1/5 of cases
Nuclear chromatin	Coarse to fine	Delicate and dispersed
Nucleoli	0–2, less prominent	1–4, often prominent
Myelodysplastic changes	Absent	May be present
MPO/Sudan black	Negative	Often positive
Chloroacetate esterase	Negative	Positive in myeloid leukemia
Nonspecific esterase	Negative	Positive in monocytoid leukemia
PAS	Often positive	Positive in about 10%–15% of cases
TdT	Frequently positive	Positive in occasional cases
Common ALL antigen (CD10)	Frequently positive	Negative
Myeloid antigens	Negative	Positive
Gene rearrangement	Frequently positive	Occasionally positive

MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.

classification divides B-cell ALL into precursor B-ALL, common ALL and pre-B ALL (4).

These stages can be distinguished simply by using CD19, C μ and surface Ig. B-precursor ALL shows only CD19, pre-B ALL expresses CD19 and C μ whereas B-ALL bears CD19 and surface Ig. However, a recent study shows that surface Ig can be occasionally demonstrated in B-precursor and pre-B ALL (13). The WHO classification defines common ALL by its expression of CD10 (4). The malignant nature of the ALL cells is determined by TdT, CD10 (common ALL antigen), and CD34 (hematopoietic

progenitor antigen). TdT is present in most cases of ALL except for B-ALL. CD10 is seen in most cases of B-cell ALL. CD34 is present in B-precursor ALL (but not in pre-B ALL) and in some cases of B-ALL.

Additional antibodies that can be helpful in classifying ALL include HLA-DR, CD20, CD22, and CD24 (Table 6.17.3). Cytoplasmic CD22 appears earlier than surface CD22 in the B-cell developmental stage and is consistently positive in B-ALL (14).

A relatively new marker, CD79a, has been routinely used to identify B cells in ALL cases at St. Jude Children's

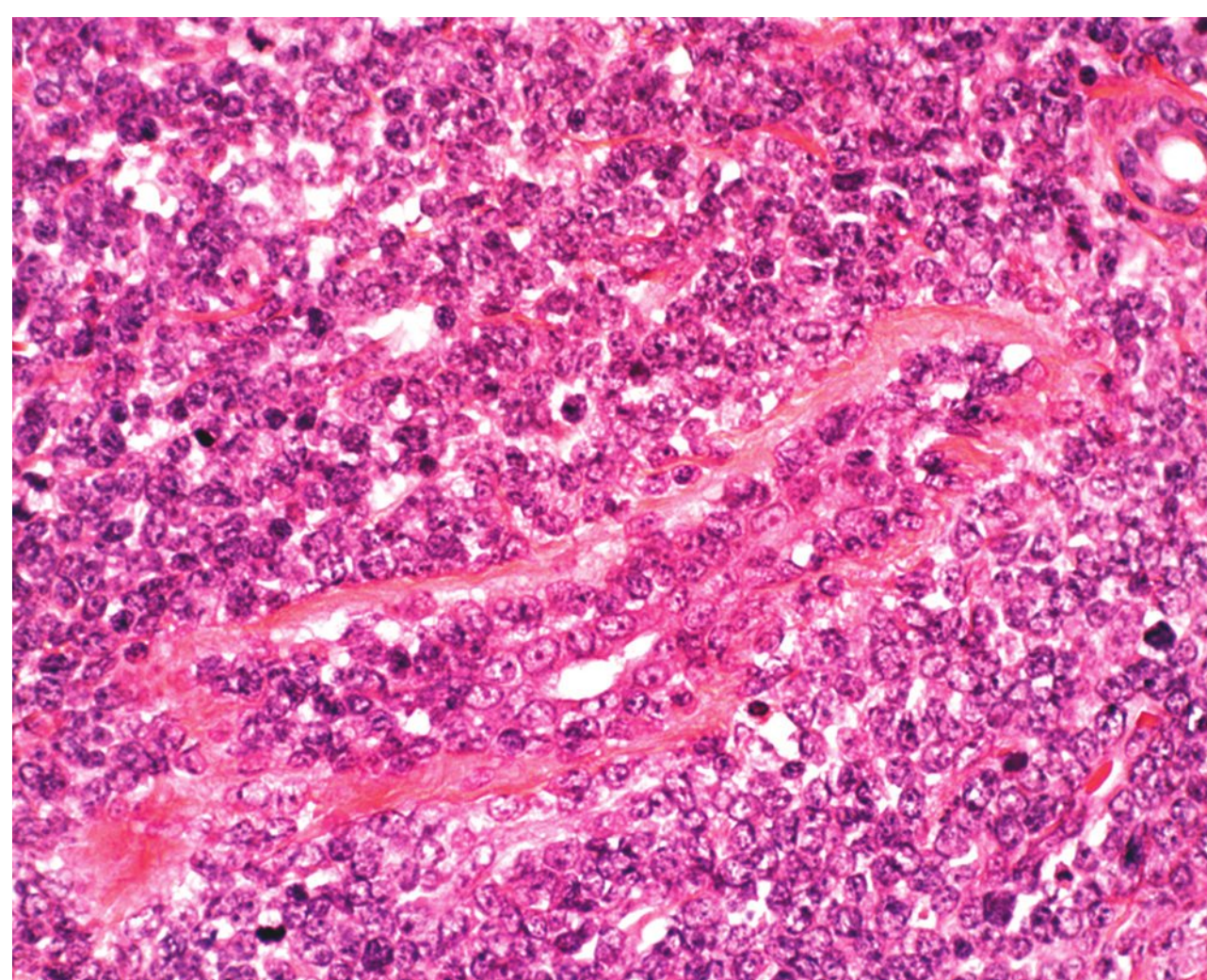


FIGURE 6.17.5 Breast biopsy of ALL case reveals ductal and periductal leukemic infiltration. Wright–Giemsa, 40× magnification.

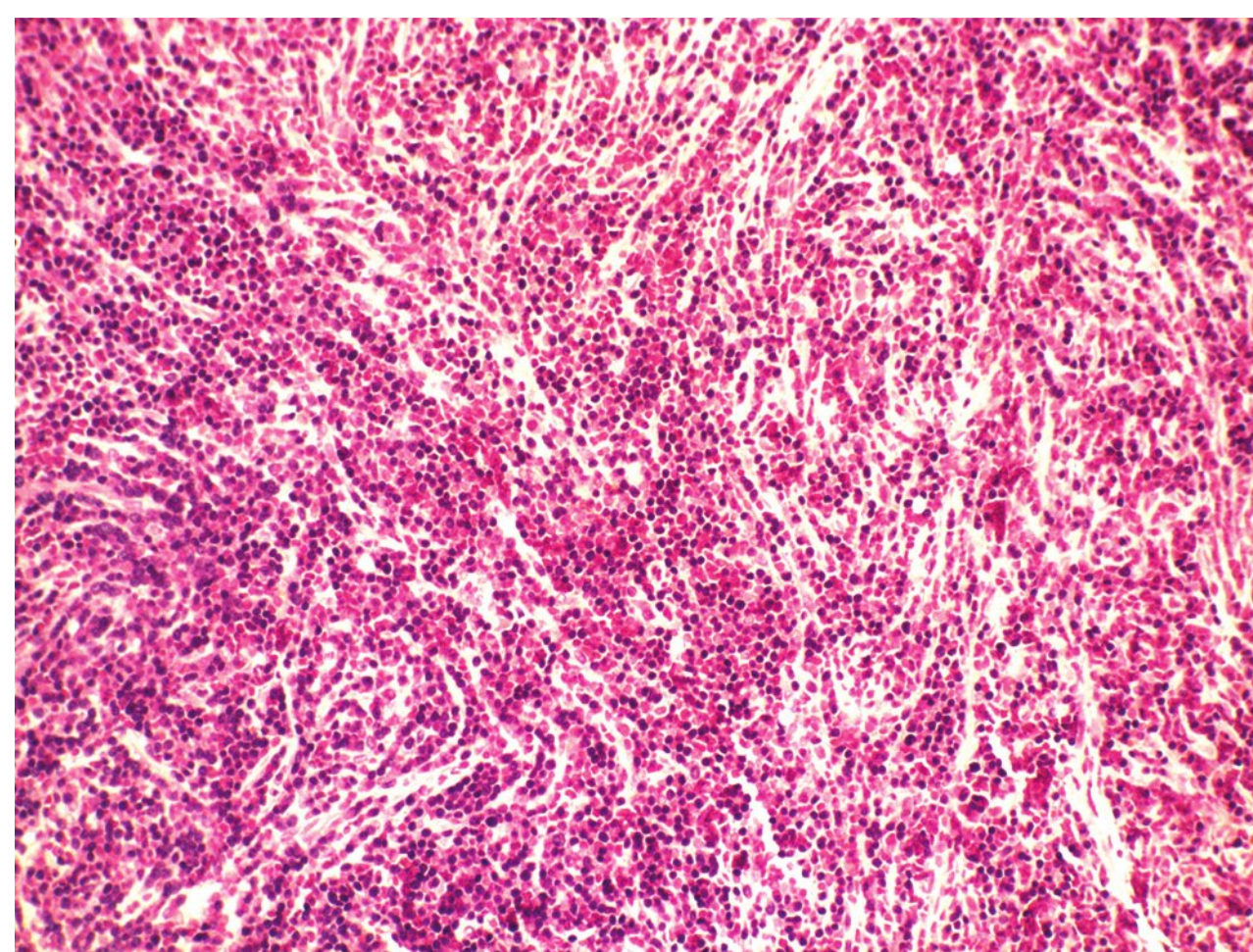


FIGURE 6.17.6 Periosteum from a case of ALL with characteristic single-file infiltration. Hematoxylin and eosin, 20× magnification.

TABLE 6.17.3			
Immunophenotypic Classification of B-Lineage ALL			
Antigens	B-precursor ALL	Pre-B ALL	B-ALL
CD10	+	+	±
CD19	+	+	+
CD20	±	±	+
Cyto-CD22	+	+	+
CD22	-	-	+
CD24	+	+	+
CD34	+	-	-
Cyto-CD79a	+	+	+
HLA-DR	+	+	+
Cyto-m	-	+	-
Surface Ig	-	-	+
TdT	+	±	-

ALL, acute lymphoblastic leukemia; Cyto-, cytoplasmic; Ig, immunoglobulin; TdT, terminal deoxynucleotidyl transferase; HLA-DR, human leukocyte antigen-DR.

Research Hospital (15). In another study, CD79a was found in the cytoplasm of B cells in most cases of ALL of different categories, including early B cell, pre-B cell, and mature B cell, as well as in common ALL. CD79b is also present in the cytoplasm of B cells in different kinds of ALL, but it is a less sensitive marker than CD79a (16).

A recent study showed that several transcription factors are frequently expressed in B-cell ALL cases with the sensitivity of 100% (FAX5), 94% (BOB.1), 97% (PU.1), and 26% (OCT-2) (17). They are more sensitive markers than CD79 (85%), CD22 (79%), and CD20 (6%) (17). The mantle cell lymphoma-associated SOX11 transcription factor is also frequently expressed in T- and B-cell lymphoblastic neoplasms (18).

Mixed phenotype ALL cases are encountered occasionally. When CD2 and CD19 are demonstrated, those cases usually represent precursor-B ALL rather than T-cell ALL (T-ALL) because Ig rearrangements are demonstrated in most of

these cases, whereas T-cell receptor (TCR) rearrangement is rarely observed (19). One or more myeloid markers can be demonstrated in as many as one fourth of children and one third of adults with ALL (11). However, a diagnosis of acute mixed phenotype leukemia should be reserved for cases with definitive evidence of both myeloid and lymphoid characteristics by immunophenotyping and genotyping (16,20). Immunophenotypically, either MPO or two monocytic markers are acceptable for a definitive myeloid lineage identification. The cases that have BCR-ABL or MLL rearrangement are designated as distinct entities in the 2008 WHO classification (4). Cases with no specific karyotype are called mixed phenotype acute leukemia, B/myeloid, not otherwise specified (NOS), which usually have poor prognosis (20).

The immunologic classification also correlates with the FAB subgroups and associates with certain cytogenetic abnormalities (Table 6.17.4) (10,11). B-precursor ALL

TABLE 6.17.4			
Correlation between Immunologic Classification, FAB Subgroups, and Cytogenetic Abnormalities			
Immunologic subgroup	FAB subgroup	Cytogenetic abnormalities	Approximate frequency
B-precursor ALL	L1, L2	t(9;22), 11q23 rearr., t(12;21)	50%
Pre-B ALL	L1, L2	t(9;22), 11q23 rearr., t(1;19)	20%
B-ALL	L3	t(8;14), t(2;8), t(8;22)	4%

ALL, acute lymphoblastic leukemia; FAB, French-American-British; rearr., rearrangement.

constitutes 50% of adult ALL and shows L1 or L2 morphology. It is associated with t(9;22), 11q23 rearrangement, and t(1;19). B-ALL is present in 4% of adult ALL and is the leukemic counterpart of Burkitt lymphoma. Therefore, it shows L3 morphology and is associated with t(8;14), t(2;8), or t(8;22). In B-ALL patients showing no L3 morphology, the cytogenetic changes may also be different, and the prognosis is worse than it is in patients with L3 morphology (10). Because many of these patients carry t(14;18), some authors have suggested that these may represent cases of follicular lymphoma progressing to a leukemic phase with blast transformation (21). However, recent studies show that many of these cases have t(4;11) translocation involving the AF4 and mixed lineage leukemia (MLL) genes (22). The immunophenotype of these cases is characterized by the absence of CD10 and coexpression of myeloid-associated markers, particularly CD15 (23).

There were several studies of ALL patients, in which L1 morphology was associated with mature immunophenotype (CD34⁺, TdT⁺, surface Ig⁺) (24). Those patients responded to the precursor B-ALL regimen and achieved complete clinical remission (24). On the other hand, cases with L3 morphology but immature immunophenotype (CD34⁺, TdT⁺, surface Ig⁺) responded to the protocol for Burkitt lymphoma. Therefore, morphology could be more specific than immunophenotyping for the distinction between L1/L2 and L3 in some cases (24).

One distinct function of immunophenotyping by flow cytometry is to distinguish regenerated lymphoblasts (hematogones) after chemotherapy of ALL versus leukemic lymphoblasts. Wells et al. (25) emphasized the assessment of the dot-plot projections (patterns) using pairs of monoclonal antibodies (CD2/CD19, CD20/CD10, CD22/CD34, HLA-DR/CD11b, CD33/CD13, and cytoplasmic TdT) combined with CD45 peridinin-chlorophyll-protein complex (perCP). In comparison with the pattern of normal lymphoblasts, they found the following aberrations in leukemic lymphoblasts: increased side scatter, increased forward scatter, decreased CD45 expression, overexpression of CD10, underexpression of CD10, absence of CD10, desynchronous CD22/CD34, decreased CD19 expression, myeloid antigen expression, and absence of CD34. Because the aberration differs in each case, such changes can be used to identify tumor cells in the bone marrow of a particular patient and is thus helpful in detecting minimal residual disease (MRD).

Chen et al. (26) identified multiple aberrations in precursor B-lymphoblastic leukemia/lymphoma by flow cytometry and suggested that these aberrations can be used for the detection of MRD. The most common aberrancies in their study were abnormal expression patterns of CD34 and/or TdT; overexpression or underexpression of CD10, CD22, and HLA-DR; underexpression of CD38 and CD45; asynchronous coexpression of mature and immature antigens (coexpression of CD20 and CD34); and cross-lineage antigen expression (26).

As mentioned before, hematogones should be distinguished from lymphoblasts, especially after chemotherapy. Hematogones can be divided into three maturation stages (27). Stage 1 hematogones express TdT, CD34,

CD10, CD19, CD22, and CD38. In stage 2, TdT and CD34 are down-regulated and CD10 is partially down-regulated, but CD20 and surface Ig start to appear. Stage 3 hematogones show the same markers as in stage 2 with strong expression of CD20 and surface Igs. The major distinction between hematogones and lymphoblasts is that the former always express a continuous and complete maturation spectrum and lack asynchronous or aberrant antigen expression, whereas neoplastic lymphoblasts often show aberrant immunophenotype (27).

Comparison between Flow Cytometry and Immunohistochemistry

A large panel of antibodies can be used in flow cytometric analysis. Flow cytometry is also able to distinguish cytoplasmic from surface staining (e.g., cytoplasmic CD22 and Cm). Therefore, this technique is superior to immunohistochemistry for the diagnosis of ALL. However, hematogones can be easily recognized with immunohistochemical stain due to direct morphologic correlation.

In the current case, the negative cytochemical staining in MPO, α -naphthyl butyrate esterase, and chloroacetate esterase but positive PAS staining is not supportive of acute myelogenous leukemia. The positive reactions to TdT, CD10, CD34, and CD19 are consistent with ALL of B-cell lineage. The presence of CD13/CD33 markers can be seen in ALL cases and it does not mean biphenotypic leukemia (6,20). In terms of stage, the absence of Cm excludes pre-B ALL, and the absence of surface Igs rules out B-ALL. Therefore, this case should be diagnosed as B-precursor ALL.

Molecular Genetics

Cytogenetics plays an important role in ALL, because it is the most powerful prognostic predictor that can be used to guide the therapeutic approach. On the basis of cytogenetic findings, childhood precursor-B ALL can be divided into three distinct subgroups (12). The low-risk group includes ALL cases with hyperdiploidy (>50 chromosomes), t(12;21), and dic(9;12). The high-risk group includes those cases with 11q23 translocations, t(9;22), and hypodiploidy (<46 chromosomes). The remaining cases, including those with t(1;19), are classified in the intermediate-risk group. However, another study showed that the poor prognosis associated with pre-B ALL is attributable to its association with the translocation t(1;19) (14). The higher frequency of t(9;22) in adult ALL as compared with childhood ALL partially accounts for the generally poor outcome in adult cases (28). For the high-risk group, the patient should be treated aggressively with early bone marrow transplantation. The low-risk patients can be treated with less toxic drugs, such as antimetabolites.

Numerical chromosome aberrations, either alone or in association with structural abnormalities, are present in about half of ALL cases. These changes can be divided into several ploidy groups, namely, low hyperdiploidy (47 to 50 chromosomes), high hyperdiploidy (>50 chromosomes or DNA index >1.15), hypodiploidy, pseudodiploidy (46 chromosomes with structural abnormalities), and gain or loss of a single chromosome (29).



There are more than 30 structural abnormalities, including translocation, deletion, inversion, isochromosome, and dicentric chromosome (dic), known to be present in ALL; the more important ones are listed in Table 6.17.5 (29–31). Among these abnormal karyotypes, t(9;22), t(v;11q23), t(12;21), t(5;14), and t(1;19) have been classified as distinct entities in the 2008 WHO scheme (4).

The Groupe Francais de Cytogénétique Hématologique found structural abnormalities in 78% of ALL cases studied (28). In recent years, most genes involved in translocations have been characterized by molecular biology (30,31). Because molecular biology techniques are usually more sensitive than karyotyping, they have become very important tools for diagnosis and prognostic prediction in cases of ALL. The Southern blotting technique has been gradually replaced by polymerase chain reaction (PCR) and reverse transcriptase (RT)-PCR techniques. In addition, the fluorescence in situ hybridization technique is able not only to detect numerical chromosome aberrations but also translocations. Protooncogenes are usually involved in chromosomal translocations. As a result, either the protooncogene is activated or a fusion transcript/chimeric protein is formed to induce tumorigenesis (30,31). The loss of tumor suppressor genes is another mechanism in the pathogenesis of ALL.

When an immunophenotype is not conclusive, Ig gene or TCR gene rearrangement should be considered (32). Most B-cell ALL cases show Ig gene rearrangement. However, most B-precursor ALL cases reveal TCR d chain gene rearrangement (33). Although Ig or TCR gene rearrangements are present in virtually all ALL cases, cross-lineage gene rearrangements occur in >90% of precursor B-ALL and in about 20% of T-ALL, so that a conclusive result may not be obtainable (34). The selection of methods is also important.

Because combinatorial diversity is relatively restricted for TCR g and TCR a rearrangements, Southern blotting is the method of choice for their detection (30). In contrast, IgH, TCR b, and TCR d show considerable junctional diversity, and PCR is preferred.

Recent studies show that gene expression profiling has a great potential in stratifying ALL cases, predicting prognosis, and guiding treatment selection (35,36). Gene profiling has also identified unique leukemia-associated markers, which can be monitored by flow cytometry for the detection of MRD (35). For instance, ALL cases with MLL rearrangements are associated with CD10–, CD24–, CD15+ (36). ALL with t(1;19) is characterized by CD10+, CD34–, CD20– Cm(6). B-ALL with t(12;21) shows CD10+, HLA-DR+, CD9–, CD20– (6).

The salient features for laboratory diagnosis of B-ALL are summarized in Table 6.17.6.

Clinical Manifestations

ALL is mainly a pediatric neoplasm with an early incidence peak at 2 to 5 years of age that represents about 80% of the childhood leukemia in the United States (29). The incidence in the pediatric group is approximately 30 cases per 1 million children younger than 15 years. However, ALL has a bimodal distribution, with a second peak around age 50 years, and a steady rise in incidence thereafter. The incidence of ALL in adults is about one third that in children. In the United States, ALL is more frequently seen in whites than in blacks (1.8:1) and in boys than in girls (1.2:1) (12).

The clinical symptoms of ALL are due to suppression of hematopoiesis in the bone marrow and, occasionally, extramedullary leukemic infiltration. The most common symptom is anemia, which manifests as pallor, weakness, and excessive tiredness. Hemorrhages, such as petechiae, ecchymoses, and epistaxis, occur in about

TABLE 6.17.5

Important Chromosomal Abnormalities and Genes Involved in ALL

Abnormality	Genes involved	Approximate incidence
*t(9;22)(q34;q11)	BCR, ABL	Adults: 30%; children: 3%
t(8;14)(q24;q32)	c-MYC, IgH	1%
t(2;8)(p12;q24)	c-MYC, IgK	<1%
t(8;22)(q24;q11)	c-MYC, IgL	<1%
*t(1;19)(q23;p13)	E2A, PBX1	5%
t(17;19)(q22;p13)	E2A, HLF	<1%
*t(5;14)(q31;q32)	IL3, IgH	<1%
*t(1;11)(p32;q23)	MLL, AF1P	<1%
*t(4;11)(q21;q23)	MLL, AF4	Infants: 60%; adults: 5%
*t(9;11)(p22;q23)	MLL, AF9	<1%
*t(12;21)(p13;q22)	TEL, AML1	Adults: <1%; children: 20%

*Karyotypes that are classified as distinct entities in the 2008 WHO scheme.
BCR, breakpoint cluster region; ABL, Ableson; c-MYC, an oncogene derived from avian myelocytomatosis virus; Ig, immunoglobulin; HLF, hepatic leukemia factor; MLL, mixed lineage leukemia; TEL, translocation-Ets-leukemia oncogene; AML1, acute myeloid leukemia 1.

TABLE 6.17.6

Salient Features for Laboratory Diagnosis of B-Cell ALL

1. TdT positive for precursor-B and pre-B ALL
2. CD10 positive in all subgroups, except for some B-ALL cases
3. HLA-DR positive in all subgroups
4. Positive markers for B-cell lineage: CD19, CD20, CD22, CD24, CD79a, PAX5, BOB.1, PU.1, and OCT-2. CD20 is frequently negative
5. Cμ positive in pre-B ALL only
6. Monoclonal surface immunoglobulin in B-ALL only
7. Immunoglobulin gene or TCR gene rearrangements

ALL, acute lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase.

two thirds of patients. Neutropenia, which may lead to a predisposition to bacterial infections, is less commonly seen. Lymphadenopathy and splenomegaly are seen in three fourths of patients, and hepatomegaly in one half of patients. In ALL cases, extramedullary involvement is frequent, including commonly the central nervous system, liver, spleen, lymph node, testis, kidney, bone, and joint (3,4). In B-LBL cases, the skin, soft tissue, bone, and lymph nodes are most frequently involved (6). However, any organ system can be affected. Unlike T-LBL cases, mediastinal involvement is infrequent.

The current cure rate is about 80% to 95% in children but only 30% to 85% in adults (4,12,37,38). This discrepancy is partly due to the higher frequency of adverse genetic aberrations (e.g., BCR-ABL1 fusion gene) and partly due to the usually higher leukocyte count or other factors present in the adult ALL population. In contrast, children aged 1 to 9 years usually have hyperdiploidy and favorable genetic changes (e.g., TEL/AML1 fusion gene). The prognosis of infants <12 months old is generally poor. This may be related to both clinical and biologic factors, such as high leukocyte counts at diagnosis, irregular or immature phenotypes, and unfavorable molecular and cytogenetic abnormalities (e.g., MLL rearrangement) (39).

The follow-up examination of bone marrow after chemotherapy for the detection of MRD has been advocated in recent years, and it has proven to be a powerful tool for the prediction of prognosis (40). There are several sensitive techniques for the detection of MRD, including flow cytometry, PCR, RT-PCR, and fluorescence in situ hybridization (25,31,41). The detection of MRD usually predicts relapse of ALL. However, some recent studies found that PCR analysis may be too sensitive, and long-term remission may be sustained in the presence of MRD detected by PCR (42). Therefore, a threshold of residual disease level should be determined or several techniques should be used to detect MRD at different time points after treatment. One study found that flow cytometric analysis at week 14 postchemotherapy was the most predictive (43).

REFERENCES

1. Head DR, Behm FG. Acute lymphoblastic leukemia and the lymphoblastic lymphomas of childhood. *Semin Diagn Pathol.* 1995;12:325–334.
2. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of acute leukemias. *Br J Haematol.* 1976;33:451–458.
3. Brunning RD, McKenna RW. *Tumors of the Bone Marrow.* Washington, DC: Armed Forces Institute of Pathology; 1994:100–142.
4. Borowitz MJ, Chan JKC. B lymphoblastic leukaemia/lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues.* 4th ed. Lyon, France: IARC Press; 2008:168–175.
5. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization Classification of Hematological Malignancies. Report of the Clinical Advisory Committee Meeting. Airlie House, Virginia, November 1997. *Mod Pathol.* 2000;13:193–207.
6. Brunning RD, Borowitz M, Matutes E, et al. Precursor B lymphoblastic leukaemia/lymphoblastic lymphoma (precursor B-cell acute lymphoblastic leukemia). In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues.* Lyon, France: IARC Press; 2001:111–114.
7. Li CY, Yam LT, Sun T. *Modern Modalities for the Diagnosis of Hematologic Neoplasms.* New York: Igaku-Shoin; 1997:7–19.
8. Kroft SH, Asplund SL, McKenna RW, et al. Haematogones in the peripheral blood of adults: a four-colour flow cytometry study of 102 patients. *Br J Haematol.* 2004;126:209–212.
9. Foon KA, Todd RF. Immunologic classification of leukemia and lymphoma. *Blood.* 1986;68:1–31.
10. Jennings CD, Foon KA. Recent advances in flow cytometry. Application to the diagnosis of hematologic malignancy. *Blood.* 1997;90:2863–2892.
11. Copelan ES, McGuire EA. The biology and treatment of acute lymphoblastic leukemia in adults. *Blood.* 1995;85:1151–1168.
12. Pui CH. Acute lymphoblastic leukemia. *Pediatr Clin North Am.* 1997;44:831–846.
13. Kansal R, Deeb G, Barcos M, et al. Precursor B lymphoblastic leukemia with surface light chain immunoglobulin restriction. *Am J Clin Pathol.* 2004;121:512–525.
14. Borowitz MJ, DiGiuseppe JA. Acute lymphoblastic leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1643–1665.
15. Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med.* 1998;339:605–615.
16. Astsaturov IA, Matutes E, Moritla R, et al. Differential expression of B29 (CD79b) and mb-1 (CD79a) proteins in acute lymphoblastic leukemia. *Leukemia.* 1996;10:769–773.
17. Nasr MR, Rosenthal N, Syrbu S. Expression profiling of transcription factors in B- or T-acute lymphoblastic leukemia/lymphoma and Burkitt lymphoma. Usefulness of PAX5 immunostaining as pan-pre-B-cell marker. *Am J Clin Pathol.* 2010;133:41–48.
18. Dictor M, Sundberg M, Warneholt J, et al. Strong lymphoid nuclear expression of SOX11 transcription factor defines lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. *Haematologica.* 2009;94:1563–1568.
19. Melnick SJ. Acute lymphoblastic leukemia. *Clin Lab Med.* 1999;19:169–186.

20. Borowitz MJ, Bene MC, Harris NL, et al. Acute leukaemia of ambiguous lineage. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:150–155.
21. Kouides PA, Phatak PD, Wang N, et al. B-cell lymphoblastic leukemia with L1 morphology and coexistence of t(1;19) and t(14;18) chromosome translocation. *Cancer Genet Cytogenet*. 1994;78:23–27.
22. Silverman LB, Sallan SE. Newly diagnosed childhood acute lymphoblastic leukemia-update on prognostic factors and treatment. *Curr Opin Hematol*. 2003;10: 290–296.
23. Frater JL, Batanian JR, O'Connor DM, et al. Lymphoblastic leukemia with mature B-cell phenotype in infancy. *J Pediatr Hematol Oncol*. 2004;26:672–677.
24. Li S, Lew G. Is B-lineage acute lymphoblastic leukemia with a mature phenotype and L1 morphology a precursor B-lymphoblastic leukemia/lymphoma or Burkitt leukemia/lymphoma? *Arch Pathol Lab Med*. 2003;127:1340–1344.
25. Wells DA, Sale GE, Shulman HM, et al. Multidimensional flow cytometry of marrow can differentiate leukemic from normal lymphoblasts and myeloblasts after chemotherapy and bone marrow transplantation. *Am J Clin Pathol*. 1998;110:84–94.
26. Chen W, Karandikar NJ, McKenna RW, et al. Stability of leukemia-associated immunophenotypes in precursor B-lymphoblastic leukemia/lymphoma. A single institution experience. *Am J Clin Pathol*. 2007;127:39–46.
27. McKenna RW, Asplund SL, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) and neoplastic lymphoblasts by 4-color flow cytometry. *Leuk Lymphoma*. 2004;45:277–285.
28. Armstrong SA, Look AT. Molecular genetics of acute lymphoblastic leukemia. *J Clin Oncol*. 2005;23:6306–6315.
29. Faderl S, Kantarjian HM, Talpaz M, et al. Clinical significance of cytogenetic abnormalities in adult acute lymphoblastic leukemia. *Blood*. 1998;91:3995–4019.
30. Macintyre EA, Delabesse E. Molecular approaches to the diagnosis and evaluation of lymphoid malignancies. *Semin Hematol*. 1999;36:373–389.
31. Thandla S, Aplan PD. Molecular biology of acute lymphocytic leukemia. *Semin Oncol*. 1997;24:45–56.
32. Crist WM, Grossi CE, Pullen DJ, et al. Immunologic markers in childhood acute lymphoblastic leukemia. *Semin Oncol*. 1985;12:105–121.
33. Yokota S, Hansen-Hagge TE, Ludwig WD, et al. Use of polymerase chain reactions to monitor minimal residual disease in acute lymphoblastic leukemia patients. *Blood*. 1991;77:331–339.
34. Szczepanski T, Pongers-Willems MJ, Langerak AW, et al. Unusual immunoglobulin and T-cell receptor gene rearrangement patterns in acute lymphoblastic leukemia. *Curr Top Microbiol Immunol*. 1999;246:205–213.
35. Pui CH, Relling MV, Downing JR. Acute lymphoblastic leukemia. *N Engl J Med*. 2004;350:1535–1548.
36. Parkin JL, Arthur DC, Abramson CS, et al. Acute leukemia associated with the t(4;11) chromosome rearrangement: ultrastructural and immunologic characteristics. *Blood*. 1982;60:1321–1331.
37. Ravindranath Y. Recent advances in pediatric acute lymphoblastic and myeloid leukemia. *Curr Opin Oncol*. 2003;15:23–35.
38. Kebriaei P, Larson RA. Progress and challenges in the therapy of adult acute lymphoblastic leukemia. *Curr Opin Hematol*. 2003;10:284–289.
39. Basso G, Rondelli R, Covezzoli R, et al. The role of immunophenotype in acute lymphoblastic leukemia of infant age. *Leuk Lymphoma*. 1994;15:51–60.
40. Moppett J, Burke GAA, Steward CG, et al. The clinical relevance of detection of minimal residual disease in childhood acute lymphoblastic leukaemia. *J Clin Pathol*. 2003;56:249–253.
41. Campena D, Coustan-Smith E. Detection of minimal residual disease in acute leukemia by flow cytometry. *Cytometry*. 1999;38:139–152.
42. Estrov Z, Freedman MGH. Detection of residual disease in acute lymphoblastic leukemia of childhood. *Leuk Lymphoma*. 1999;33:47–52.
43. Coustan-Smith E, Sancho J, Hancock ML, et al. Clinical importance of minimal residual disease in childhood acute lymphoblastic leukemia. *Blood*. 2000;96:2691–2696.

CASE 18

T-Lymphoblastic Leukemia/Lymphoma

CASE HISTORY

A 10-year-old boy was admitted to the hospital because of intermittent cough, dyspnea, progressive wheezing, and orthopnea for approximately 1 month. He was treated for asthma to no avail. Chest x-ray examination revealed a large anterior mediastinal mass with tracheal deviation. Pericardial effusion was also detected. Physical examination found cervical, supraclavicular, and axillary lymphadenopathy. However, the liver and spleen were not palpable. Hematologic workup revealed a total leukocyte

count of 512,000/mL with 15% lymphocytes, 12% neutrophils, and 72% blasts. His hematocrit was 35% and platelet count 95,000/mL. The blood chemistry profile was unremarkable except for an extremely high level of lactate dehydrogenase (LDH) (960 U/L).

After admission, the patient was immediately treated with combined chemotherapy and radiation therapy. However, the size of the mediastinal mass and peripheral lymphadenopathy remained unchanged after treatment. He became increasingly hypoxic and bradycardic and died 5 days after admission.

At autopsy, a large mediastinal mass was found that encased the roots of the aorta, pulmonary artery, and superior vena cava. The tumor also compressed the trachea and invaded the epicardium. The pericardial fluid contained a large number of blasts.

FLOW CYTOMETRIC FINDINGS

The peripheral blood showed 0% CD2, 1% surface CD3, 54% cytoplasmic CD3, 5% CD3/CD4, 10% CD3/CD8, 6% CD5, 98% CD7, 2% CD10, 1% CD19, 3% CD25, 97% CD34, and 85% terminal deoxynucleotidyl transferase (TdT) (Fig. 6.18.1).

DISCUSSION

T-lymphoblastic leukemia/lymphoma was previously divided into T-lymphoblastic lymphoma (LBL) and T-acute lymphoblastic leukemia (ALL) in the old classifications. However, this new designation has been adopted by both the Revised European-American Lymphoma (REAL) classification (1) and the World Health Organization (WHO) classification (2), because T-LBL and T-ALL are morphologically identical and clinically similar.

LBL was originally called Sternberg sarcoma and was first described by Smith et al. (3) as a T-cell lymphoma derived from thymic lymphocytes. Barcos and Lukes (4) further defined its morphologic and clinical characteristics and considered it a distinct clinical immunopathologic entity. LBL was also called convoluted T-cell lymphoma because its nuclei are convoluted in most cases. In the Working Formulation of non-Hodgkin Lymphoma, LBL was divided into the convoluted and nonconvoluted subtypes (5).

In children, B-ALL is composed of approximately 85% of ALL cases, whereas T-ALL is identified in about 15% of ALL patients (6,7). In adult patients, T-ALL accounts for 25% of ALL cases (8).

Morphology

The typical morphologic feature of LBL is the presence of a “starry sky” histologic pattern due to the presence of numerous tangible-body macrophages as a result of accelerated apoptosis (Fig. 6.18.2). Mitosis is also prominent (Table 6.18.1). This histologic pattern is indistinguishable from that of Burkitt lymphoma. However, these two entities can be differentiated by their cytology (Table 6.18.2). Cells from LBL are intermediate in size with scanty cytoplasm, which shows no vacuolation in imprints. Their nuclei

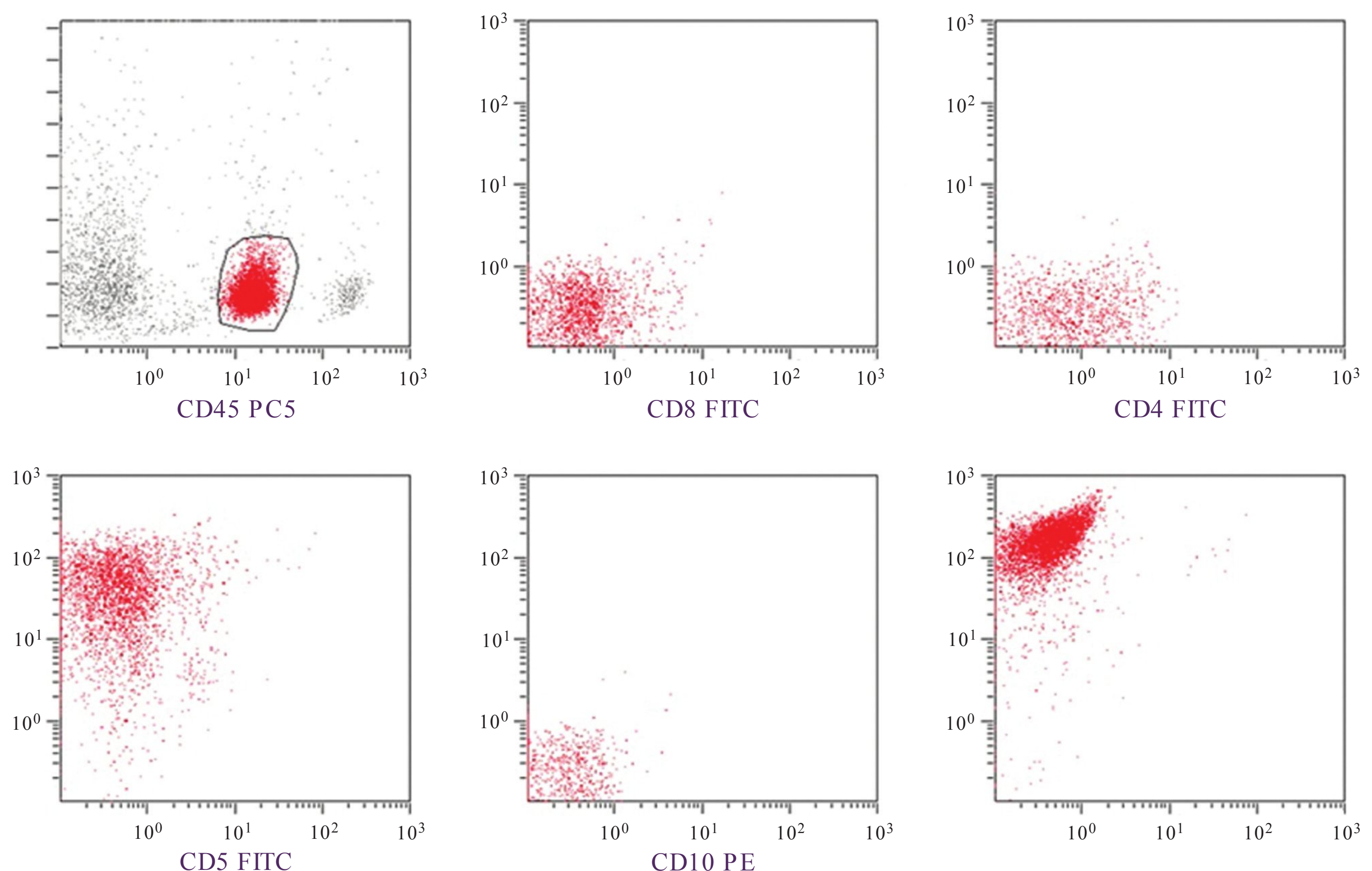


FIGURE 6.18.1 Flow cytometric histograms show that the tumor cells react only to CD7 and CD34 with low percentages of CD3/CD4 and CD3/CD8. CD5, CD10, and CD19 are negative. SS, side scatter; PC5, phycoerythrin–cyanin 5; ECD, phycoerythrin–Texas Red; PE, phycoerythrin; RD1, rhodamine; FITC, fluorescein isothiocyanate.

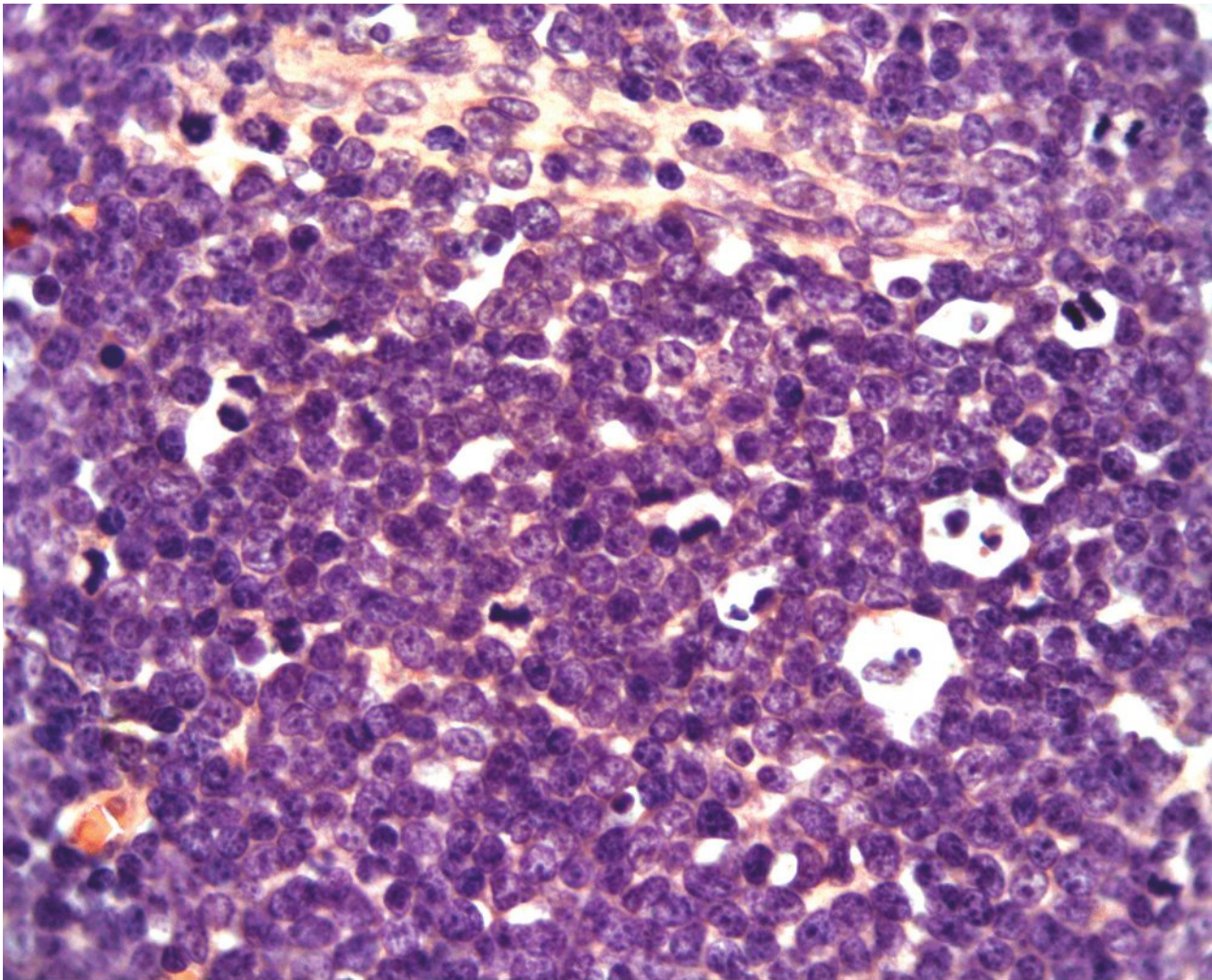


FIGURE 6.18.2 Lymph node biopsy shows a “starry sky” histologic pattern with tangible-body macrophages and mitotic figures in the vacuoles. Hematoxylin and eosin, 60× magnification.

are usually convoluted, containing dusky chromatin and inconspicuous nucleoli. However, LBL may also manifest as a nonconvoluted form or an atypical pleomorphic form (9). In touch preparations, LBL reveals an L1/L2 morphology (10). Cells from Burkitt lymphoma are medium sized with abundant pyroninophilic cytoplasm, which is deeply basophilic and often vacuolated in imprint preparations. Their nuclei are round or ovoid, containing clumped chromatin and multiple nucleoli in tissue sections (11). Touch

TABLE 6.18.1	
Characteristic Morphologic Features of LBL	
Histologic pattern	Diffuse lymphoid infiltration on a “starry sky” background
Cytology	Small-to-intermediate size, scanty cytoplasm, convoluted nuclei, dusky chromatin, and inconspicuous nucleoli
Specific features	“Starry sky” pattern and convoluted nuclei with a high mitotic rate

preparations of a lymph node may show the L3 morphology with more immature-looking chromatin than in tissue sections. In case of doubt, immunophenotyping is helpful; LBL is predominantly of T-cell origin, but Burkitt lymphoma is exclusively of B-cell type.

LBL and T-ALL are considered the tissue and leukemic phases of the same disease. Their distinction is rather arbitrary, depending on the distribution of the tumor cells: LBL is mainly in the soft tissue, but ALL is predominantly in the blood and bone marrow (12). However, LBL may have a leukemic phase with bone marrow involvement. On the other hand, ALL may also involve lymph nodes, particularly the mediastinal lymph node. The arbitrary cutoff point for the distinction of these two entities is 25% of lymphoblasts in the bone marrow, above which is designated ALL; otherwise, it is considered LBL with bone marrow involvement (13). Immunophenotyping does not help in

TABLE 6.18.2		
Comparison of LBL and Burkitt Lymphoma		
	LBL	Burkitt lymphoma
High incidence group	Children	Children
Clinical presentation	Mediastinal mass	Jaw or abdominal lesion
Mitotic rate	High	High
“Starry sky” pattern	Less prominent	More prominent
Cell size	Small to intermediate	Intermediate to large
Cytoplasm	Scanty, pale blue, no vacuoles	Abundant, dark blue, vacuolated
Nuclear shape	Usually convoluted	Round to ovoid
Chromatin pattern	Finely speckled	Clumped
Nucleoli	Inconspicuous	Multiple, distinct
FAB type	L1/L2	L3
Phenotype	Predominantly T cell	Exclusively B cell
Cytogenetic aberration	Not specific	t(8;14), t(8;22), or t(2;8)
EBV related	No	Yes

EBV, Epstein–Barr virus; FAB, French-American-British.

their distinction, because their phenotypes are essentially identical. The lymphoblasts in T-ALL show either L1 or L2 morphology (Fig. 6.18.3), whereas L3 is always of B-cell lineage.

Cytochemically, the lymphoblasts are periodic acid-Schiff positive, but are negative for myeloperoxidase, as well as for specific and nonspecific esterases. T lymphoblasts may also show focal acid phosphatase staining (2).

Immunophenotype

The traditional immunologic classification divides T-cell ALL into four immunophenotypes (7). The pre-T-cell phenotype expresses only CD7, cytoplasmic CD3, and TdT without other T-cell antigens. The early cortical phenotype shows CD2, CD5, CD7, and strong TdT. The late cortical phenotype reveals CD1a, CD2, CD5, CD7, and dual CD4/CD8 with minimal surface CD3. The medullary phenotype shows CD2, CD3, CD5, CD7, and segregated CD4 or CD8. TdT is not commonly expressed in this phenotype. Cytoplasmic CD3 is expressed in all stages (14). The above-mentioned four stages have been changed to pro-T-, pre-T-, cortical T-, and medullary (mature) T-cell stages in the current literature (Table 6.18.3) (8). However, ALL immunophenotypes frequently do not correlate with recognized stages of normal lymphocyte maturation and may not conform to a maturation arrest model. In fact, the common diagnostic feature for T-cell neoplasms is either loss or aberrant expression of T-cell antigens (7,15). The U.S.–Canadian Consensus Recommendation Group indicated that the coexpression of cytoplasmic CD3 and TdT/CD34 alone is diagnostic for T-ALL (16). The phenotypic features of adult T-ALL are similar to those of childhood T-ALL, but HLA-DR and CD10 are more frequently positive in adults than in children (14).

On the basis of therapeutic response and prognosis, most authors consider it unnecessary to divide T-ALL into multiple subtypes. The general consensus is either

to divide it into pre-T-cell and T-cell ALL or not to divide T-ALL into any subtypes (10,17,18).

However, individual markers may be used to predict the prognosis. Two study groups found that T-ALL cases that expressed CD10 had better prognosis than those without CD10 expression in terms of remission rate and event-free survival (19,20). CD3 positivity associated with an abnormal karyotype, in contrast, was reported to be a significant adverse risk factor (21). Another report showed statistically significant correlation between the CD2 antigen expression frequency and event-free survival (6). The comparison of ALL cases with different maturation phenotypes showed no statistical significance in terms of therapeutic response and prognosis (22,23). The coexpression of myeloid markers in T-ALL cases was reported to show a worse prognosis than those without (24,25). This conclusion, however, was not confirmed by other studies (26). Current opinion is that myeloid markers may be associated with unfavorable prognosis in adult but not childhood ALL (8). CD117 is usually not expressed in T-ALL cases; the expression of this marker was found to be associated with lower complete response rates (27).

Most cases of LBL are of thymic origin, with approximately one half of the T-cell cases corresponding to common thymocytes and one fourth each to early thymocytes and late thymocytes (13,28). Immature B-cell (pre-pre-B cell and pre-B cell), mature B-cell, and natural killer (NK)-cell types have also been reported (9,12,13,28–33). Sheibani et al. (29) divided LBL into five groups: LBL with T-cell phenotype (T-LBL), T-LBL with expression of common ALL antigen (CALLA or CD10), T-LBL with expression of NK cell-associated antigens, LBL with pre-B-cell phenotype, and B-LBL (Table 6.18.4). These immunophenotypes show some clinical correlations, such as the absence of mediastinal mass in the pre-B-cell and B-cell phenotypes and the aggressive clinical course seen in NK-associated antigen phenotypes (29,31,32). However, the NK-associated antigen phenotype is considered to be blastic NK-cell lymphoma/leukemia by other studies (34). Skin involvement is more frequently seen in the CALLA-positive phenotype (30,35), and skin as well as lytic bone lesions occur more often in the immature B-cell phenotype (9,12,30).

Among all markers, TdT is most useful because it is present in almost all cases of LBL (except for mature B-cell type) and is seldom, if ever, seen in other lymphomas (Fig. 6.18.4) (12,29). In the REAL classification, TdT positivity is only listed in precursor T- and precursor B-LBL and/or leukemia (1). Therefore, a positive TdT reaction may exclude the diagnosis of Burkitt lymphoma. The reactivities of T-cell monoclonal antibodies depend on the stage of thymocytes to which the tumor cells are related (36). Generally, CD2 is consistently positive in all study series, and CD1a is specific for the common thymocyte stage (12,28,29). The reactivities of CD4 and CD8 are usually used as the criteria for stage identification: early thymocytes are CD4–, CD8–; common thymocytes are CD4+, CD8+; and late thymocytes are CD4+, CD8– or CD4–, CD8+.

CD38, an antigen present in thymocytes and plasma cells, showed a high positive rate in cases of LBL from

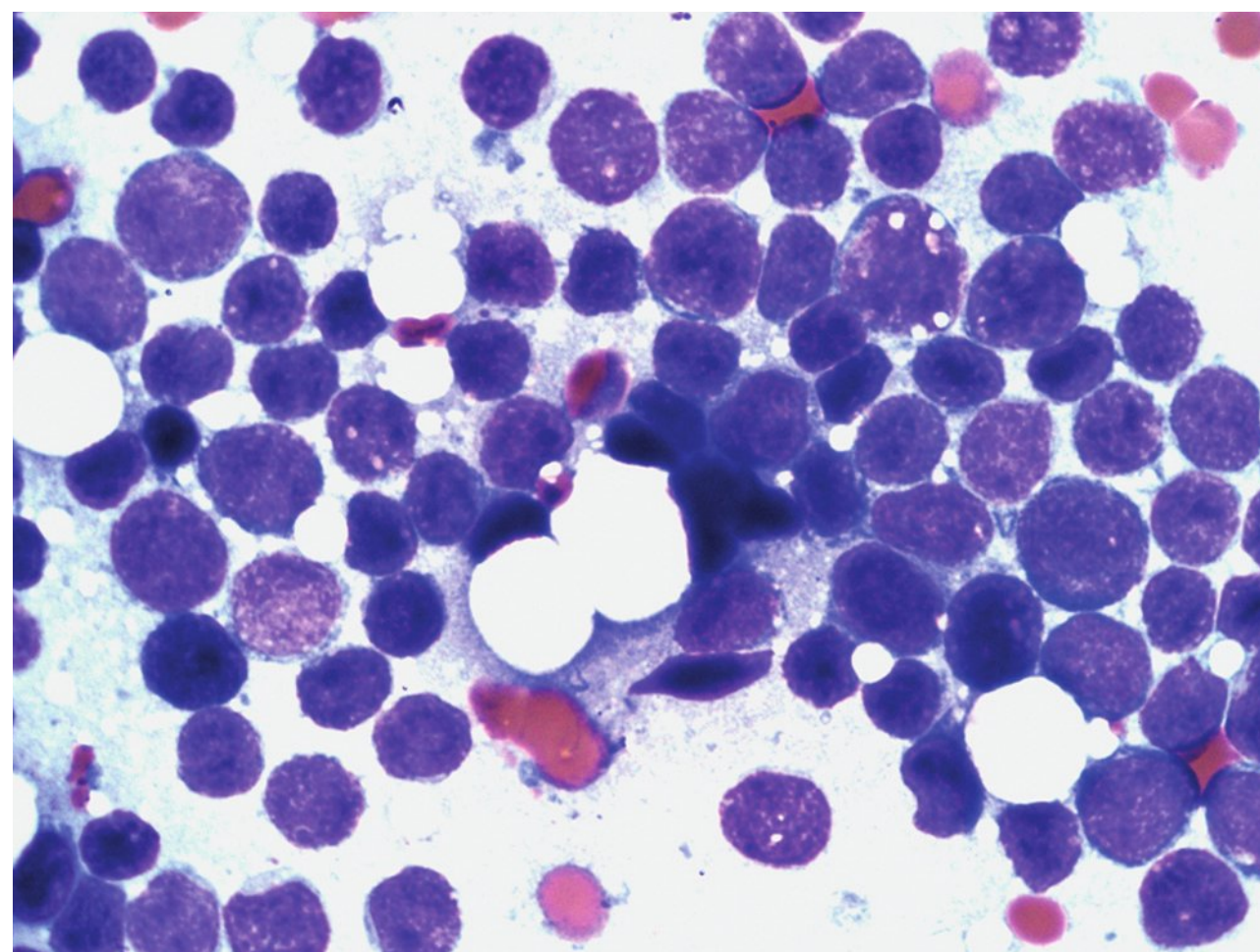


FIGURE 6.18.3 Bone marrow aspirate shows many lymphoblasts with different sizes, immature chromatin pattern, and inconspicuous nucleoli. A few lymphoblasts reveal vacuolated cytoplasm. Wright–Giemsa, 100× magnification.

TABLE 6.18.3

Immunophenotype of T-Cell ALL/LBL

	CD1a	CD2	cCD3	sCD3	CD4	CD5	CD7	CD8	TdT
Pro-T	-	-	+	-	-	-	+	-	+
Pre-T	-	+	+	-	-	+	+	-	+
Cortical T	+	+	+	-	+	+	+	+	+
Medullary T	-	+	+	+	±	+	+	±	±

TdT, terminal deoxynucleotidyl transferase; c, cytoplasmic; s, surface.

most studies; thus, it is a useful marker for LBL (12,13,28). CD71, the transferrin receptor antigen, was present in 6 of 15 cases and 3 of 11 cases of LBL, respectively, in two separate series (12,29). In two study series, all cases of LBL were negative for CD25, which represents the α subunit of the interleukin-2 receptor (28,29), but the β subunit of interleukin-2 is widely expressed among T-LBLs (36).

When the thymic element in a thymoma is examined by flow cytometry, the phenotype may mimic LBL. However,

in most circumstances, these two entities can be distinguished morphologically. Cytokeratin stain should be positive for thymoma and negative for LBL, although focally positive staining for keratin has been reported in a small group of LBL cases (37).

In rare conditions, pre-B-cell LBL may present as a solitary bone tumor mimicking Ewing sarcoma (37). This group of tumors may express antigens that are positive for Ewing sarcoma, including CD99 and vimentin. To make the

TABLE 6.18.4

Immunophenotypes of LBL

Immunophenotype	Special feature
I. T-cell LBL	Mediastinal mass (86%)
1. Early thymocytes	
TdT+, CD1a-, CD2+, CD3-,* CD4-, CD5+, CD7+, CD8-	
2. Common thymocytes	
TdT+, CD1a+, CD2+, CD3+, CD4+, CD5+, CD7+, CD8+	
3. Late thymocytes	
TdT+, CD1a-, CD2+, CD3+, CD4±, CD5+, CD7-, CD8±	
II. T-cell LBL with CALLA	Skin involvement
Thymocyte phenotype with positive CALLA (CD10)	
III. T-cell LBL with NK antigens†	Nonwhite female predominant, aggressive clinical course
Thymocyte phenotype with positive CD16 and/or CD57	
IV. Pre-B-cell LBL	Bone or skin lesions
TdT-, CD10+, HLA-DR+, C μ +, CD9+, CD24+, T-antigens	No mediastinal mass
V. B-cell LBL	No mediastinal mass
TdT-, HLA-DR+, SIg+, T-antigen	

*Cytoplasmic CD3 is present in all stages.

†Other studies include this entity into NK-cell lymphoma.

CALLA, common acute lymphoblastic leukemia antigen; NK, natural killer; TdT, terminal deoxynucleotidyl transferase.

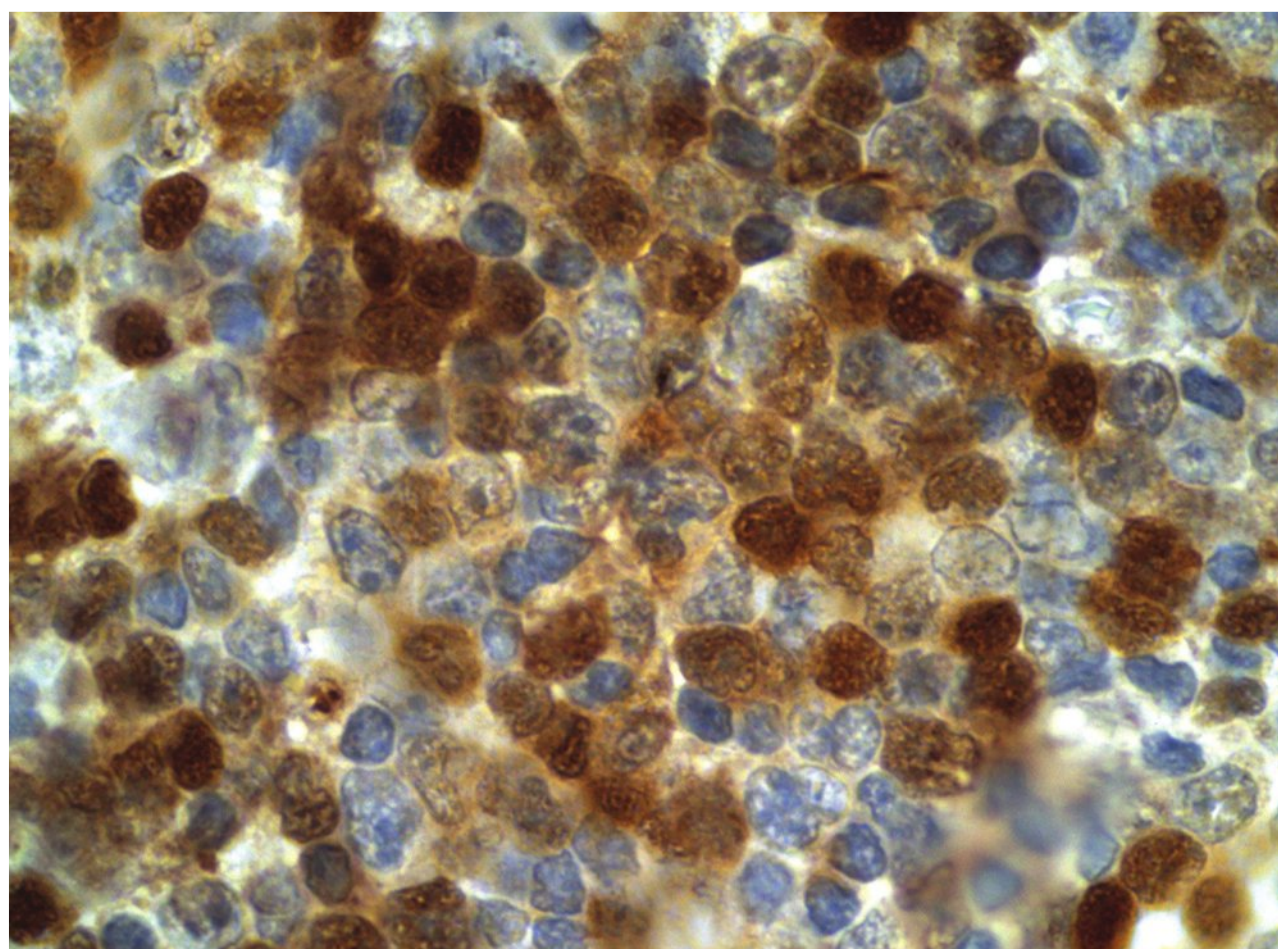


FIGURE 6.18.4 Lymph node biopsy of LBL shows positive staining for TdT. Immunoperoxidase, 100× magnification.

matter even worse, B-LBL may be CD45 and CD20 negative, which may mislead to the exclusion of lymphoma. In those cases, however, CD79a and CD19 are positive; thus, these antibodies should be included for differential diagnosis.

For immunohistochemical staining, CD43 (MT-1) is very reliable marker for immature hematopoietic neoplasms; it is 100% positive in T-LBL, 80% in B-LBL, and almost 100% in myeloid sarcomas (38). The reaction of CD45RO (UCHL-1) in LBL cases is controversial (39,40). CD3 is often present in T lymphoblasts (9), but most cases show localization to the cytoplasm (40). In some cases of LBL, CD3 was coexpressed with CD79a, a B-cell marker (41). However, all these cases showed T-cell receptor (TCR) gene rearrangements, indicating their T-cell lineage (42). Recently, the mantle cell lymphoma-associated SOX11 transcription factor has been found to be strongly expressed in most B- and T-ALL/LBL cases, but its expression is independent of cyclin D1 (43). Since the blastoid form of mantle cell lymphoma may mimic T-LBL, the presence of SOX11 staining should be interpreted with caution.

In the current case, the clinical history is typical for the patient's age and the presence of a mediastinal mass. The diagnosis, however, was established by flow cytometric analysis of the peripheral blood, which showed an immunophenotype of precursor-T-lymphoblastic leukemia/lymphoma. Because of the presence of all the immature cell markers and because both CD4 and CD8 were negative, the tumor was at the stage of early thymocyte. The mediastinal tumor tissue obtained at autopsy showed a typical starry sky pattern with numerous mitotic figures, confirming the diagnosis of LBL. The compression of the large blood vessels and the trachea as well as the invasion of the pericardium finally led to the death of the patient.

Comparison of Flow Cytometry and Immunohistochemistry

For the examination of peripheral blood and bone marrow, flow cytometry is superior to immunohistochemistry because all the antibodies mentioned above are readily available for this technique. Therefore, flow cytometry not

only makes the diagnosis but also pinpoints the maturation stage of the tumor cells. However, for a mediastinal tumor, immunohistochemistry is much more reliable to distinguish between LBL and a thymoma. A TdT staining is most helpful, as LBL is the only lymphoma that expresses TdT. Cytokeratin stain, in contrast, is most helpful for the diagnosis of thymoma.

Molecular Genetics

Although T-ALL and B-ALL share a few cytogenetic abnormalities, there are some characteristic changes that are specific to T-ALL. In terms of numerical changes, hyperdiploid karyotype is more frequently demonstrated in B-ALL, whereas pseudodiploid karyotype (normal chromosomal number with structural abnormality) is more often seen in T-ALL. Also, near-tetraploid (chromosomes >65) is more frequently seen in T-ALL than in B-ALL (6). Because hyperdiploidy is usually associated with favorable outcome, whereas pseudodiploidy and near-tetraploidy carry poor outcome, the prognosis of T-ALL is generally worse than that of B-ALL.

The structural changes in T-ALL are characterized by the frequent involvement of the TCR β locus at 7q32-36, the TCR α locus at 14q11, and the TCR γ locus at 7p14-15 (6,8,44-46). The involvement of the first two loci comprises approximately 20% of all T-ALL cases (6). The most common structural change is translocation, which leads to a dysregulation of the partner gene under the control of the enhancer of the TCR gene. In rare cases, *inv*(14)(q11q32) and a tumor-associated lymphocyte gene (TAL1) disruption may be present. This disruption occurs through an interstitial deletion between the SIL (SCL interrupting locus) and the 5' untranslated region of SCL, resulting in a fusion transcript SIL/SCL (6). TAL1 can also be dysregulated by a microscopic deletion of the short arm of chromosome 9, *del*(9p), resulting in loss of the tumor suppressor gene CDKN2A (2).

The important translocations involving the 14q11 are *t*(1;14), *t*(8;14), *t*(10;14), and *t*(11;14). Those involving the 7q32-36 include *t*(1;7), *t*(7;9), *t*(7;11), and *t*(7;19). The structural changes in T-ALL are summarized in Table 6.18.5. The NOTCH1 gene, which is involved in T-ALL with *t*(7;9) translocation, is of particular importance (46,47). NOTCH1 point mutations, insertions, and deletions producing aberrant increases in NOTCH1 signaling are frequently present in both childhood and adult T-ALL (46,47). It is hypothesized that aberrant NOTCH1 signaling plays an important role in the pathogenesis of T-lymphoblastic leukemia/lymphoma, and NOTCH1 inhibitors (such as γ -secretase inhibitor) may prove to be effective for the treatment of this entity (46,47). Unlike B-ALL, none of the aberrant karyotypes is listed as distinct entities in the 2008 WHO classification.

TCR γ -chain or δ -chain gene rearrangement can be detected in most cases of T-cell ALL, and polymerase chain reaction has been used to monitor minimal residual disease in this leukemia with a certain degree of success (48,49). The salient features for laboratory diagnosis of T-ALL are listed in Table 6.18.6.

TCR gene rearrangement can be demonstrated in most cases of LBL. This analysis is helpful to distinguish LBL from Burkitt lymphoma, which shows immunoglobulin heavy-chain gene rearrangement and thymoma, which

TABLE 6.18.5		
Structural Changes of Chromosomes Associated with T-ALL		
Abnormality	Gene involved	Approximate frequency (%)
t(1;14)(p32;q11)	TAL1, TCRa/d	1–3
t(8;14)(q24;q11)	c-MYC, TCRa/d	2
t(10;14)(q24;q11)	HOX11, TCRa/d	5–10
t(11;14)(p15;q11)	LMO1, TCRa/d	1
t(11;14)(p13;q11)	LMO2, TCRa/d	5–10
inv(14)(q11q32)	TCL1, TCRa/d	<1
t(1;7)(p33;q35)	SCL, TCRb	<1
t(1;7)(q34;q34)	LCK, TCRb	<1
t(7;9)(q35;q34)	TAL2, TCRb	<1
t(7;9)(q34;q34.3)	NOTCH1, TCRb	<1
t(7;19)(q35;p13)	LYL1, TCRb	<1
t(7;11)(q35;p13)	TTG2, TCRb	<1
t(11;19)(q23;p13)	MLL, ENL	<1

ALL, acute lymphoblastic leukemia; TAL, tumor-associated lymphocyte gene; TCR, T-cell receptor; cMYC, an oncogene derived from avian myelocytomatosis virus; HOX, homeobox gene; TCL, T-cell leukemia; LYL, lymphoblastic leukemia gene; MLL, mixed lineage leukemia gene.

reveals germline in genotyping. However, genotyping is not entirely lineage specific, because up to 30% of B-LBLs may show TCRg gene rearrangements and 10% to 25% of T-LBLs show heavy-chain gene rearrangements (50).

Karyotypic studies in LBL are not as extensive as in T-ALL. In general, these two entities share the same chromosomal aberrations. In a study of 17 cases of T-LBL, 16 had karyotypic abnormalities, most commonly involving 14q11, 7q35, and 7p15, which are similar to those seen in T-ALL (51). Three cases showed a unique t(9;17)(q34;q23) translocation that has not been seen in ALL cases.

TABLE 6.18.6	
Salient Features for Laboratory Diagnosis of T-ALL	
1. Most cases are positive for TdT.	
2. Pediatric cases are frequently negative for CD10 and HLA-DR.	
3. CD7 is frequently the only positive T-cell marker, but all T-cell markers can be present.	
4. Dual CD4/CD8 positivity is more common than other CD4/CD8 combinations.	
5. TCR gene rearrangement is present in most cases.	
6. Translocation frequently involves TCRb at 7q32-36, TCRa d at 14q11, or TCRg at 7p14–15.	

ALL, acute lymphoblastic leukemia; TdT, terminal deoxynucleotidyl transferase; TCR, T-cell receptor.

A specific syndrome showing LBL, eosinophilia, and myeloid hyperplasia and/or malignancy has been reported in fewer than 10 cases, all of them associated with t(8;13)(p11;q11) translocation (52). Recently, a pediatric case with the same syndrome was reported without cytogenetic abnormality detected by conventional karyotyping and by molecular cytogenetic techniques (53). Pre-B LBL is frequently associated with t(1;19) and E2X/PBX (9,50). The t(9;22) translocation is commonly associated with precursor B-cell LBL (54). No LBL-associated karyotype is classified as a distinct entity in the 2008 WHO scheme.

The recent investigations of T-ALL/LBL with gene expression profiling (GEP) show a great potential of this technique. GEP can help to stratify patients into different subtypes in relation to their therapeutic responses and prognosis (55–57). One study showed that GEP identified prognostically important leukemia subtypes, including T-ALL, E2A-PBX1, BCR-ABL, TEL-AML1, MLL rearrangement, and hyperdiploid >50 chromosomes (55). Another study found that GEP may help to distinguish peripheral and lymphoblastic T-cell lymphomas (56). Gene expression studies also identified five multistep molecular pathways that play an important role in the pathogenesis of T-ALL, involving HOX11, HOX11L2, TAL1 plus LMO1/2, LYL1 plus LMO2, and MLL/ENL (46,58). LYL1+ signature corresponds to pro-T stage, HOX11+ to early cortical stage, and TAL1 to late cortical thymocyte stage (58). Patients with HOX11-positive lymphoblasts have an excellent prognosis, while cases with TAL1 and LYL1-associated ALL are at high risk of early failure (58).

The salient features for laboratory diagnosis of LBL are summarized in Table 6.18.7.

TABLE 6.18.7

Salient Features for Laboratory Diagnosis of LBL

1. Special marker: LBL is the only lymphoma that expresses TdT.
2. Most T-cell antigens are positive, corresponding to different thymocyte stages.
3. Thymocyte staging depends on the reactions of CD4 and CD8.
Early thymocyte: CD4–, CD8–
Common thymocyte: CD4+, CD8+
Late thymocyte: CD4±, CD8±
4. TCR antigens are positive.
5. CD38 and CD71 are frequently positive.
6. Immunohistochemistry: CD43 for both T- and B-cell tumors, CD20 and CD79a for B-cell tumors.
7. Small percentages of LBL show B-cell or NK-cell phenotype.
8. TCR gene rearrangement is frequently demonstrated.

NK, natural killer; TdT, terminal deoxynucleotidyl transferase; TCR, T-cell receptor.

Clinical Manifestations

LBL is most frequently seen in male adolescents and accounts for one third of childhood non-Hodgkin lymphomas (10,59). Pediatric patients with a typical T-LBL usually present with a mediastinal mass, which is frequently associated with supradiaphragmatic (cervical, supraclavicular, or axillary) lymphadenopathy. The mediastinal mass may cause airway obstruction, superior vena cava syndrome, pericardial effusions, and pleural effusions (59,60). Constitutional symptoms are seen in about 30% of patients. In adult patients, however, the presentation is frequently extramediastinal, mainly abdominal (61), or subcutaneous lesions (9,29). Other sites of involvement include head and neck, lung, liver, pleura, pericardium, peritoneum, and testes (60). Rare cases involving the uterus and breasts have recently been reported (62,63). Although bone marrow is frequently involved, pancytopenia or circulating blasts are unusual in LBL cases (60).

LBL is a highly aggressive tumor; patients usually die within 1.5 years (64). The most reliable prognostic factors are serum LDH levels and Ann Arbor disease staging (60). In the low-risk subgroup, characterized by Ann Arbor stage I to III disease or Ann Arbor stage IV disease with serum LDH < 1.5 times normal, >90% of patients achieved 5-year disease-free survival (60). When patients are in Ann Arbor stage IV with bone marrow or central nervous system involvement or stage IV with other extranodal site involvement and a high serum LDH level, only 20% of patients achieve a 5-year disease-free survival. A recent evaluation of T-LBL cases showed that the 5-year disease-free survival was 24% (65). Most of the earlier literature suggested that B-LBL involved bone marrow frequently and

had a particularly aggressive clinical course (65). However, one study found that bone marrow involvement was less frequently seen in B-LBL than in T-LBL, and the former group was more likely than the latter to achieve a complete remission (66). The unfavorable outcome of B-LBL is frequently associated with certain cytogenetic abnormalities, such as t(1;19) and t(9;22) (54).

In ALL, the influence of the cell lineage is just the opposite of LBL. The T-ALL cases usually have worse prognosis than the B-ALL cases.

In childhood T-ALL, the clinical presentation is characterized by older age, nonwhite race, male gender, higher white cell count (>50,000/mL), mediastinal mass, marked hepatosplenomegaly, lymphadenopathy, and central nervous system involvement (6,7). Older age, high white cell count, and organomegaly are considered high-risk factors. Therefore, patients with T-ALL usually have worse prognosis than those with B-ALL. Some reports indicate that pre-T phenotype is worse than other T-cell phenotypes, but other studies showed no differences between these two groups (10). If pre-T ALL is defined as lack of E-rosette formation but reactive with T-cell antibodies, then it is not surprising that the absence of CD2 (E-rosette receptor) is associated with adverse clinical outcome (67).

However, the use of highly intensive treatment protocols in recent years has improved the outcome of T-ALL patients. For instance, a comparative study by the Children's Cancer Group showed that the probability of 3-year survival for patients with T-ALL increased from 56% in studies conducted between 1978 and 1983 to 65% in the period between 1983 and 1989 and to 78.8% between 1989 and 1993 (6). The Dana-Farber Cancer Institute study of 125 patients with childhood T-ALL treated between 1981 and 1995 showed the 5-year event-free survival rate to be 75% ± 4% (68).

REFERENCES

1. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
2. Borowitz M, Chan JKC. T lymphoblastic leukaemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al. eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:176–178.
3. Smith JL, Barker CR, Clein GP, et al. Characterization of malignant mediastinal lymphoid neoplasm (Sternberg sarcoma) as thymic in origin. *Lancet*. 1974;1:74–77.
4. Barcos MP, Lukes RJ. Malignant lymphoma of convoluted lymphocytes—a new entity of possible T-cell type. In: Sinks LF, ed. *Conflicts in Childhood Cancer: An Evaluation of Current Management*. Vol. 4. New York, NY: Alan R. Liss; 1975:147–178.
5. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas. *Cancer*. 1982;49:2112–2135.

6. Uckun FM, Sensel MG, Sun L, et al. Biology and treatment of childhood T-lineage acute lymphoblastic leukemia. *Blood*. 1998;91:735–746.
7. Jennings CD, Foon KA. Recent advances in flow cytometry. Application to the diagnosis of hematologic malignancy. *Blood*. 1997;90:2863–2892.
8. Han X, Bueso-Ramos CE. Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma and acute biphenotypic leukemias. *Am J Clin Pathol*. 2007;127:528–544.
9. Grogan T, Spier C, Wirt DP, et al. Immunologic complexity of lymphoblastic lymphoma. *Diagn Immunol*. 1986;4:81–88.
10. Head DR, Behm FG. Acute lymphoblastic leukemia and the lymphoblastic lymphomas of childhood. *Semin Diagn Pathol*. 1995;12:325–334.
11. Brownell MD, Sheibani K, Battifora H, et al. Distinction between undifferentiated (small noncleaved) and lymphoblastic lymphoma. An immunohistologic study on paraffin-embedded, fixed tissue sections. *Am J Surg Pathol*. 1987;11:779–787.
12. Cossman J, Chused TM, Fisher RI, et al. Diversity of immunophenotype of lymphoblastic lymphoma. *Cancer Res*. 1983;43:4486–4490.
13. Bernard A, Boumsell L, Reinherz EL, et al. Cell surface characterization on malignant T-cells from lymphoblastic lymphoma using monoclonal antibodies. Evidence of phenotypic differences between malignant T-cells from patients with acute lymphoblastic leukemia and lymphoblastic lymphoma. *Blood*. 1981;57:1105–1110.
14. Deegan MJ. Membrane antigen analysis in the diagnosis of lymphoid leukemias and lymphomas. Differential diagnosis, prognosis as related to immunophenotype and recommendation for testing. *Arch Pathol Lab Med*. 1989;113:606–618.
15. Melnick SJ. Acute lymphoblastic leukemia. *Clin Lab Med*. 1999;19:169–186.
16. Borowitz MJ, Bray R, Gascoyne R, et al. U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry. Data analysis and interpretation. *Cytometry*. 1997;30:236–244.
17. Copelan EA, McGuire EA. The biology and treatment of acute lymphoblastic leukemia in adults. *Blood*. 1995;85:1151–1168.
18. Pui CH. Acute lymphoblastic leukemia. *Pediatr Clin North Am*. 1997;44:831–846.
19. Shuster JJ, Falletta JM, Puller DJ, et al. Prognostic factors in childhood T-cell acute lymphoblastic leukemia. A Pediatric Oncology Group study. *Blood*. 1990;75:166–173.
20. Dowell BL, Borowitz MJ, Boyett JM, et al. Immunologic and clinicopathologic features of common acute lymphoblastic leukemia antigen-positive childhood T-cell leukemia. A Pediatric Oncology Group study. *Cancer*. 1987;59:2020–2026.
21. Pui CH, Behm FG, Singh B, et al. Heterogeneity of presenting features and their relation to treatment outcome in 120 children with T-cell acute lymphoblastic leukemia. *Blood*. 1990;75:174–179.
22. Crost WM, Shuster JJ, Falletta J, et al. Clinical features and outcome in childhood T-cell leukemia-lymphoma according to stage of thymocyte differentiation. A Pediatric Oncology Group study. *Blood*. 1988;72:1891–1897.
23. Uckun FM, Gaynon P, Sensel M, et al. Clinical features and treatment outcome of childhood T-lineage acute lymphoblastic leukemia according to the apparent maturational stage of T-lineage leukemic blasts. A Children's Cancer Group study. *J Clin Oncol*. 1997;15:2214–2221.
24. Wiersma SR, Ortega J, Sobel E, et al. Clinical importance of myeloid-antigen expression in acute lymphoblastic leukemia of childhood. *N Engl J Med*. 1991;324:800–808.
25. Kurec AS, Belair P, Stefanu C, et al. Significance of aberrant immunophenotypes in childhood acute lymphoblastic leukemia. *Cancer*. 1991;67:3081–3086.
26. Uckun FM, Sather HN, Gaynon P, et al. Clinical features and treatment outcome of children with myeloid antigen positive acute lymphoblastic leukemia. A report from the Children's Cancer Group. *Blood*. 1997;90:28–35.
27. Tsao AS, Kantarjian HM, Thomas D, et al. C-kit receptor expression in acute leukemias: association with patient and disease characteristics and with outcome. *Leuk Res*. 2004;28:373–378.
28. Hollema H, Poppema S. T-lymphoblastic and peripheral T-cell lymphomas in the northern part of The Netherlands. An immunologic study of 29 cases. *Cancer*. 1989;64:1624–1628.
29. Sheibani K, Nathwani BN, Winberg CD, et al. Antigenically defined subgroups of lymphoblastic lymphoma. Relationship to clinical presentation and biologic behavior. *Cancer*. 1987;60:183–190.
30. Link MP, Ropper M, Dorfman RF, et al. Cutaneous lymphoblastic lymphoma with pre-B markers. *Blood*. 1983;61:838–841.
31. Swerdow SH, Habeshaw JA, Richards MA, et al. T-lymphoblastic lymphoma with Leu-7 positive phenotype and unusual clinical course. A multiparameter study. *Leuk Res*. 1985;9:167–173.
32. Sheibani K, Winberg CD, Burke JS, et al. Lymphoblastic lymphoma expressing natural killer cell-associated antigens. A clinicopathologic study of six cases. *Leuk Res*. 1987;11:371–377.
33. Schwob VS, Weiner L, Hudes G, et al. Extranodal non-T-cell lymphoblastic lymphoma in adults. A report of two cases. *Am J Clin Pathol*. 1988;90:602–605.
34. Oshima K. Leukemia and lymphoma of natural killer lineage cells. *Int J Hematol*. 2003;78:18–23.
35. Borowitz MJ, Croker BP, Metzger RS. Lymphoblastic lymphoma with the phenotype of common acute lymphoblastic leukemia. *Am J Clin Pathol*. 1983;79:387–391.
36. Rosolen A, Nakanishi M, Poplack DG, et al. Expression of interleukin-2 receptor β subunit in hematopoietic malignancies. *Blood*. 1989;73:1968–1972.
37. Ozdemirli M, Ranburg-Smith JC, Hartmann DP, et al. Precursor B-lymphoblastic lymphoma presenting as a solitary bone tumor and mimicking Ewing's sarcoma. A report of four cases and review of the literature. *Am J Surg Pathol*. 1998;22:795–804.
38. Quintanilla-Martinez L, Zukerberg LR, Ferry JA, et al. Extradural tumors of lymphoid or myeloid blasts. The role of immunohistology in diagnosis and classification. *Am J Clin Pathol*. 1995;104:431–433.
39. Kurec AS, Cruz VE, Barrett D, et al. Immunophenotyping of acute leukemias using paraffin-embedded tissue sections. *Am J Clin Pathol*. 1990;93:502–509.
40. Strikler JG, Kurtin PJ. Mediastinal lymphoma. *Semin Diagn Pathol*. 1991;8:2–13.
41. Pillozzi E, Pulford K, Jones M, et al. Co-expression of CD79a (JCB117) and CD3 by lymphoblastic lymphoma. *J Pathol*. 1998;186:140–143.
42. Pillozzi E, Muller-Hermelink HD, Falini B, et al. Gene rearrangements in T-cell lymphoblastic lymphoma. *J Pathol*. 1999;188:267–270.
43. Dictor M, Ek S, Sundberg M, et al. Strong lymphoid nuclear expression of SOX11 transcription factor defines

- lymphoblastic neoplasms, mantle cell lymphoma and Burkitt's lymphoma. *Haematologica*. 2009;94:1563–1568.
44. Thandla S, Aplan PD. Molecular biology of acute lymphocytic leukemia. *Semin Oncol*. 1997;24:45–56.
 45. Macintyre EA, Delabesse E. Molecular approaches to the diagnosis and evaluation of lymphoid malignancies. *Semin Hematol*. 1999;36:373–389.
 46. Graux C, Cools J, Michaus L, et al. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. *Leukemia*. 2006;20:1496–1510.
 47. Pear WS, Aster JC. T cell acute lymphoblastic leukemia/lymphoma: a human cancer commonly associated with aberrant NOTCH1 signaling. *Curr Opin Hematol*. 2004;11:426–433.
 48. Yakota S, Hansen-Hagge TE, Ludwig WD, et al. Use of polymerase chain reactions to monitor minimal residual disease in acute lymphoblastic leukemia patients. *Blood*. 1991;77:331–339.
 49. Taylor JJ, Rowe D, Williamson IK, et al. Detection of T-cell receptor g chain V gene rearrangements using the polymerase chain reaction. Application to the study of clonal disease cells in acute lymphoblastic leukemia. *Blood*. 1991;77:1989–1995.
 50. Medeiros LJ, Bagg A, Cossman J. Molecular genetics in the diagnosis and classification of lymphoid neoplasms. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. 2nd ed. Philadelphia, PA: W. B. Saunders; 1995:58–97.
 51. Kaneko Y, Frizzera G, Shikano T, et al. Chromosomal and immunophenotypic patterns in T cell acute lymphoblastic leukemia (T-ALL) and lymphoblastic lymphoma (LBL). *Leukemia*. 1989;3:886–892.
 52. Inhorn RC, Aster JC, Roach SA, et al. A syndrome of lymphoblastic lymphoma, eosinophilia, and myeloid hyperplasia/malignancy associated with t(8;13)(p11;q11). Description of a distinctive clinicopathologic entity. *Blood*. 1995;85:1881–1887.
 53. Lamb LS Jr, Neuberg R, Welsh J, et al. T-cell lymphoblastic leukemia/lymphoma syndrome with eosinophilia and acute myeloid leukemia. *Cytometry B Clin Cytom*. 2005;658:37–41.
 54. Knowles DM. Lymphoblastic lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:915–951.
 55. Yeoh EJ, Ross ME, Shurtleff SA, et al. Classification, subtype discovery, and prediction of outcome in pediatric acute lymphoblastic leukemia by gene expression profiling. *Cancer Cell*. 2002;1:133–143.
 56. Martinez-Delgado B, Melendez B, Cuadros M, et al. Expression profiling of T-cell lymphomas differentiates peripheral and lymphoblastic lymphomas and defines survival related genes. *Clin Cancer Res*. 2004;10:4971–4982.
 57. Chiaritti S, Li X, Gentleman R, et al. Gene expression profile of adult T-cell acute lymphocytic leukemia identifies distinct subsets of patients with different response to therapy and survival. *Blood*. 2004;103:2771–2778.
 58. Ferrando AA, Look AT. Gene expression profiling in T-cell acute lymphoblastic leukemia. *Semin Hematol*. 2003;40:274–280.
 59. Murphy SB. Classification, staging and end results of treatment of childhood non-Hodgkin's lymphoma. Dissimilarities from lymphomas in adults. *Semin Oncol*. 1980;7:332–339.
 60. Picozzi VJ, Coleman CN. Lymphoblastic lymphoma. *Semin Oncol*. 1990;17:96–103.
 61. Mazza P, Bertini M, Macchi S, et al. Lymphoblastic lymphoma in adolescents and adults. Clinical, pathological and prognostic evaluation. *Eur J Cancer Clin*. 1986;22:1503–1510.
 62. Lyman MD, Neuhauser TS. Precursor T-cell acute lymphoblastic leukemia/lymphoma involving the uterine cervix, myometrium, endometrium, and appendix. *Ann Diagn Pathol*. 2002;6:125–128.
 63. Valkiani E, Savage DG, Pile-Spellman E, et al. T-cell lymphoblastic lymphoma presenting as bilateral multinodular breast masses: a case report and review of the literature. *Am J Hematol*. 2005;80:216–222.
 64. Nathwani BN, Diamond LW, Winberg CD, et al. Lymphoblastic lymphoma. A clinicopathologic study of 95 patients. *Cancer*. 1981;48:2347–2357.
 65. The Non-Hodgkin's Lymphoma Classification Project. A clinical evaluation of the International Lymphoma Study Group Classification of non-Hodgkin's lymphoma. *Blood*. 1997;89:3909–3918.
 66. Soslow RA, Baergen RN, Warnke RA. B-lineage lymphoblastic lymphoma is a clinicopathologic entity distinct from other histologically similar aggressive lymphomas with blastic morphology. *Cancer*. 1999;85:2648–2654.
 67. Borowitz MJ, DiGituseppe JA. Acute lymphoblastic leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1643–1665.
 68. Goldberg JM, Silverman LB, Levy DE, et al. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. *J Clin Oncol*. 2003;21:3616–3622.

CASE 19

Chronic Lymphocytic Leukemia of B-Cell Lineage

CASE HISTORY

A 66-year-old man was found to have an elevated leukocyte count with lymphocytosis on a routine physical examination. The hematologic workup at that time showed a total leukocyte count of 20,200/mL with 41% neutrophils and 53% lymphocytes. The hematocrit was 44% and platelet count 301,000/mL. The patient denied having fever, weight loss, night sweats, and fatigue. He had occasional episodes of upper respiratory infection in the past year. A fine-needle biopsy of the preauricular lymph node 1 year ago showed reactive lymphoid hyperplasia, probably secondary to his ear infection at that time.

Physical examination of the patient revealed no lymphadenopathy and no hepatosplenomegaly. Further laboratory study showed normal levels of lactate dehydrogenase (120 IU/L) and beta-2 microglobulin (1.8 mg/L). Immunoglobulin quantitation demonstrated low levels of IgA (32 mg/dL) and IgM (<18 mg/dL).

After flow cytometric analysis of the peripheral blood followed by a bone marrow biopsy, a diagnosis of chronic lymphocytic leukemia (CLL) was established. However, because the patient had no lymphadenopathy, anemia, or thrombocytopenia, the disease was considered to be at Rai stage 0/Binet stage A. The patient was, therefore, not treated and was monitored by the hematology/oncology service with periodic physical and laboratory examination.

In the subsequent 4 years, the patient had several episodes of upper respiratory infection and one episode of rectal bleeding. A colonoscopic examination found no abnormality. The bleeding was considered to be due to his hemorrhoid. A follow-up laboratory examination revealed a total leukocyte count of 28,900/mL with 78% lymphocytes and 17% neutrophils. His hematocrit was 43.2%, hemoglobin 14.7 g/dL, and platelets 269,000/mL. Immunoglobulin quantitation remained at the same levels as that examined 4 years ago.

FLOW CYTOMETRIC FINDINGS

CD5 98%, CD19 85%, CD5/CD19 85%, CD23 87%, CD20 78%, CD20/CD38 10%, CD19/k 80%, CD19/l 2%, FMC-7 10%, CD10 2% (Fig. 6.19.1).

DISCUSSION

CLL of B-cell lineage (B-CLL) is the most common leukemia in Western countries, accounting for 40% of all leukemias in adults (1). In the United States, the annual incidence is about 5.17 cases per 100,000 persons (2). On the

contrary, the incidence of CLL in Asia is low, especially in India, China, and Japan (1). For instance, the incidence in Japan is 2.5% of all adult leukemias, compared with 38% in Denmark (3). The explanation of this marked geographic difference is unclear, but it is the only leukemia type that has not been associated with occupational exposure to radiation (3). Some recent epidemiological studies suggested an association of CLL with ionizing radiation, but it was not conclusive because of very small numbers of CLL cases included or lack of quantitative data (4).

Morphology

The original requirement for a peripheral lymphocyte count in CLL was 125,000/mL (5). The current criteria of the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) are a peripheral lymphocyte count of 5,000/mL for the duration of at least 3 months and the clonality of the circulating B lymphocytes is confirmed by flow cytometry as monoclonal (6,7). The IWCLL scheme allows for the diagnosis of CLL with lower lymphocyte counts in patients with lymphadenopathy or organomegaly, cytopenia, or disease-related symptoms (6). Patients with lower than 5,000/mL lymphocyte count and without the above clinical manifestations are considered to have monoclonal B-lymphocytosis (MBL) by some but low-stage CLL by others (7). MBL is considered a putative precursor of CLL in some studies (4).

Bone marrow examination is not a mandatory requirement for the diagnosis but it helps to distinguish CLL from peripheral lymphocytosis caused by infections, such as infectious mononucleosis, pertussis, and toxoplasmosis, which do not involve bone marrow.

Most cases of CLL show small mature-appearing lymphocytes in the peripheral blood, bone marrow, and, occasionally, internal organs. These lymphocytes usually have a small rim of cytoplasm and a nucleus with prominent chromatin clumping (snickerdoodle-like), but without discernible nucleoli (Fig. 6.19.2). The presence of smudge (basket) cells is another characteristic of CLL. A Mayo Clinic study found that the percentage of smudge cells inversely correlated with vimentin expression and that a higher percentage of smudge cells is present in patients with the mutated immunoglobulin heavy chain gene (8).

A small percentage of CLL cases are designated CLL mixed cell type because of the presence of small and large lymphocytes in the peripheral blood (9,10). When the prolymphocytes are composed of 10% to 55% of the cell population, the condition is termed CLL/prolymphocytic leukemia (CLL/PLL). When more than 55% prolymphocytes are present, the diagnosis becomes PLL. The differences between CLL, CLL/PLL, and PLL are summarized in Table 6.19.1 (10). In approximately two thirds of CLL/PLL patients, the

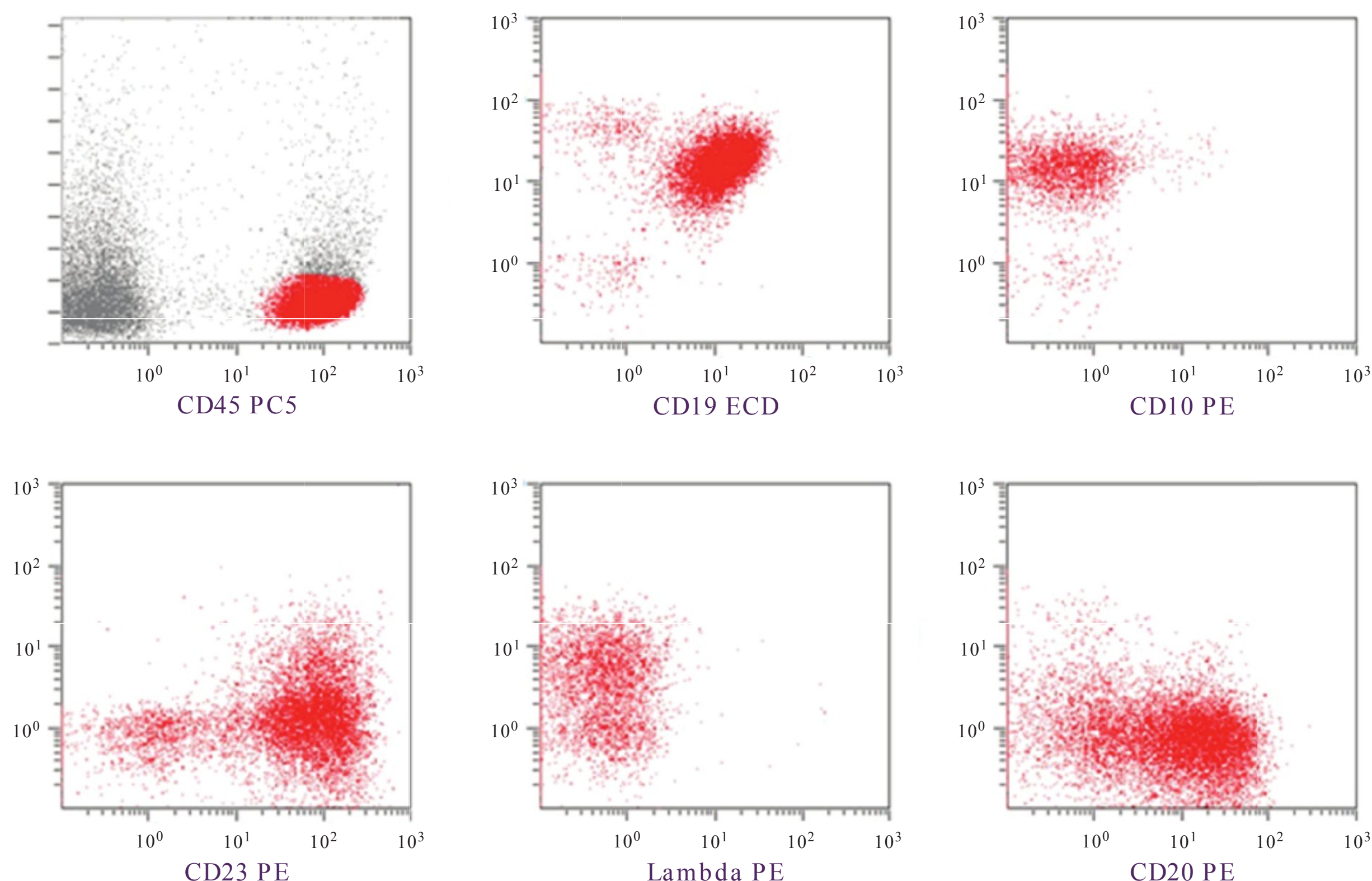


FIGURE 6.19.1 Flow cytometric histograms show dual CD19/CD5 staining and positive reactions to CD20, CD23, and k. FMC-7 is partially positive, and CD10 and CD38 are negative. The negative CD38 result predicts a favorable prognosis. SS, side scatter; FITC, fluorescein isothiocyanate; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; ECD, phycoerythrin–Texas Red.

disease may evolve and transform into PLL, which is called prolymphocytoid transformation (9,11). In the remaining patients with CLL/PLL, transformation may never occur.

Another better known transformation is Richter syndrome, which is the development of diffuse large B-cell

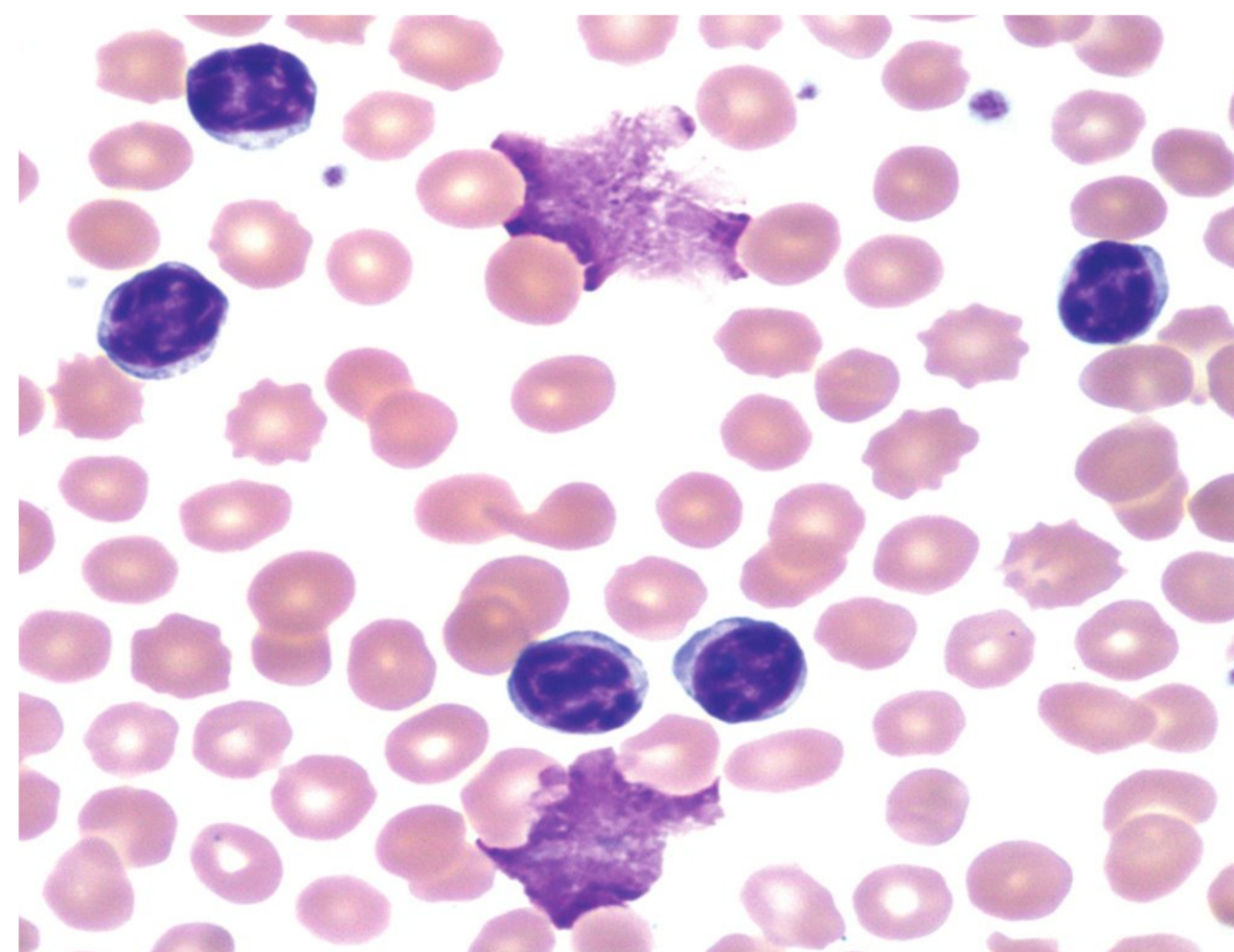


FIGURE 6.19.2 Peripheral blood smear reveals five CLL cells with prominent chromatin clumping (snickerdoodle-like) and two smudge (basket) cells. Wright–Giemsa, 150× magnification.

lymphoma in about 3% to 10% of CLL cases (Figs. 6.19.3 and 6.19.4) (12). A minority of CLL cases transforms into Hodgkin lymphoma. Extranodal Richter syndrome, involving the central nervous system, eye, gastrointestinal tract, nose, skin, face, bone or bronchus, has also been reported (13). In rare occasions, CLL can also transform into acute lymphoblastic leukemia, myeloma, and paraimmunoblastic variant of B-CLL/small lymphocytic lymphoma (3,14,15).

In the bone marrow, several infiltration patterns can be seen in CLL (14,15). A nodular pattern is discrete, densely packed collections of lymphoid cells with infiltrating margins (Fig. 6.19.5); an interstitial pattern shows CLL cells intermingled with normal hematopoietic cells and fat vacuoles. When the hematopoietic elements and fat vacuoles are largely replaced by the neoplastic cells, it is called the diffuse pattern (Figs. 6.19.6 and 6.19.7). A diffuse infiltration pattern is usually associated with disease progression and poor prognosis (16). A paratrabeular infiltration is seldom seen in CLL. Bone marrow biopsy is not only helpful in predicting the prognosis, but also useful in evaluating the therapeutic effect and determining the etiology of cytopenia (e.g., leukemic infiltration, autoimmune cytopenia, or aplastic anemia) (17).

In the lymph node, the normal architecture is effaced by diffuse proliferation of uniformly small round cells with variable numbers of plasmacytoid cells. Proliferation centers are present in at least 90% of cases (see Case 21) (15,18).

TABLE 6.19.1			
Comparison between CLL, CLL/PLL, and PLL			
	CLL	CLL/ PLL	PLL
Leukocyte count ($\times 10^9/L$)	5–120	Intermediate	>100
% of prolymphocytes	<10	10–55	>55
Splenic involvement	Less frequent	Intermediate	Constant
Lymph node involvement	Frequent	Intermediate	Seldom
Surface Ig stain	Dim	Biphasic	Bright
CD5 positivity	Constant	Biphasic	<30%
Mouse RBC rosette formation	Constant	Constant	<30%
FMC-7 positivity	<20%	<20%	Constant
Cytogenetic abnormalities	13q-, 11q-, +12	13q-, 11q-, +12	14q+

CLL, chronic lymphocytic leukemia; RBC, erythrocyte; Ig, immunoglobulin; PLL, prolymphocytic leukemia.

Immunophenotype

Because most CLL cells are morphologically indistinguishable from normal mature small lymphocytes, marker studies are important for a definitive diagnosis. Although there are many surface markers for CLL, a minimum of four markers is usually sufficient to make the diagnosis.

The first one is a monoclonal surface immunoglobulin pattern, which is almost exclusively composed of IgM-k or IgM/IgD-k. The surface immunoglobulin staining is characteristically dim, representing a low density. However, the total cellular immunoglobulin of a CLL cell is equivalent to that of most normal B cells because they contain increased cytoplasmic immunoglobulin (19).

In 10% to 20% of CLL cases, the surface immunoglobulin is too low to be detectable (20); therefore, a B-cell marker

(e.g., CD19 or CD20) should be used to identify the B-cell lineage. The intensity of CD20 is also dim in CLL cases, whereas the staining of CD19 is brighter.

The third and most specific marker for CLL is a T-cell marker, CD5, which is present in almost all cases of CLL. A dual staining for CD5 and a B-cell marker (CD19 or CD20) should be performed to ensure that those CD5-positive cells are not the reactive T cells. Because mantle cell lymphoma also expresses a dual CD19 (CD20)/CD5 phenotype, CD23 should be used to distinguish these two entities. CLL is positive but mantle cell lymphoma is negative for this marker (21). Mantle cell lymphoma can also be differentiated from CLL by its brighter fluorescence of the surface immunoglobulin, its larger size than CLL cells, and the presence of cyclin D1 staining. PLL may also show dual CD19 (CD20)/CD5 staining in one third of cases (9).

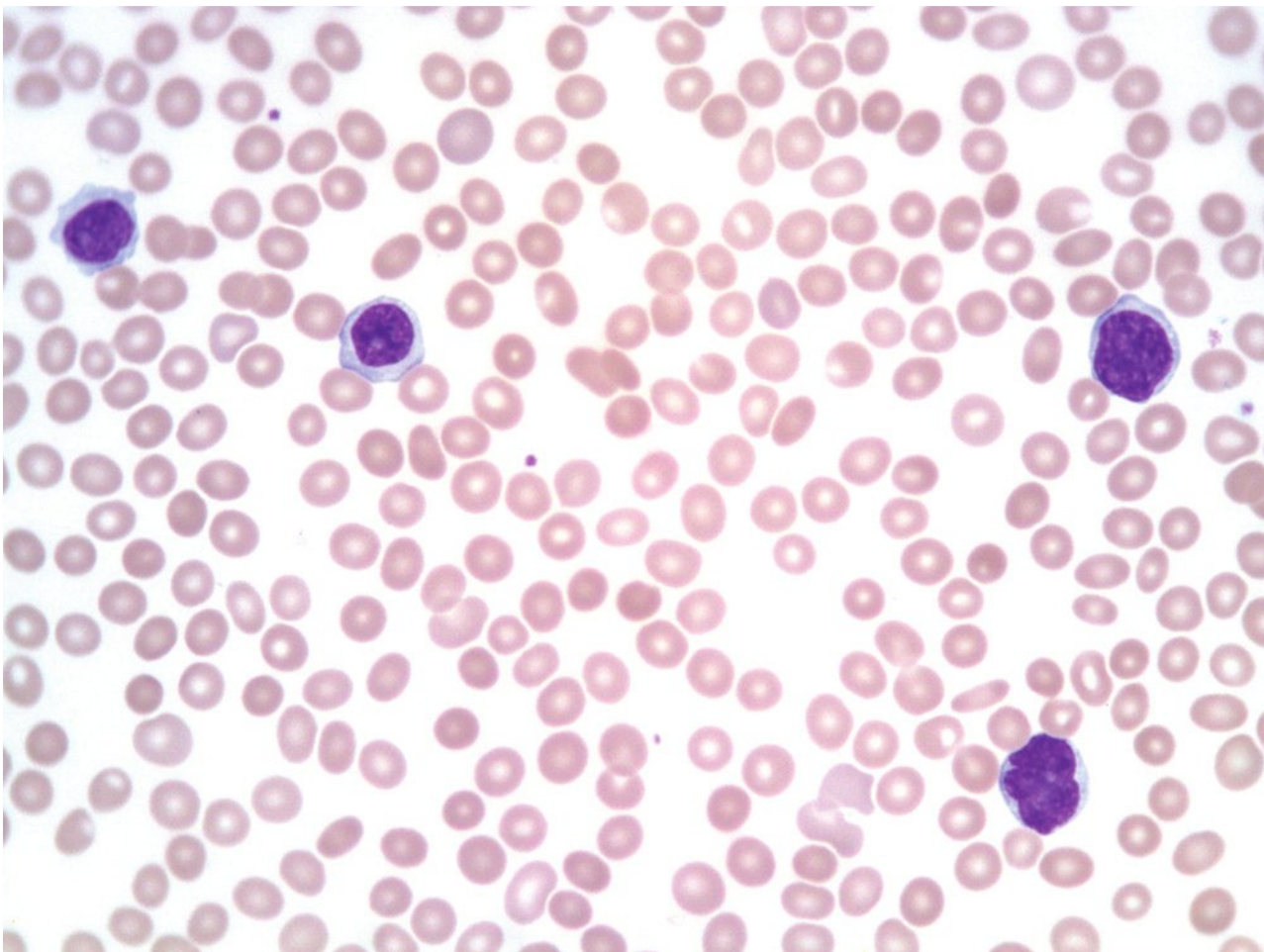


FIGURE 6.19.3 Peripheral blood smear from a CLL case shows two large lymphoid cells at the right side of the field with convoluted nucleus and prominent nucleoli. The leukemic cells carry a complex karyotype, representing Richter transformation. Wright–Giemsa, 60× magnification.

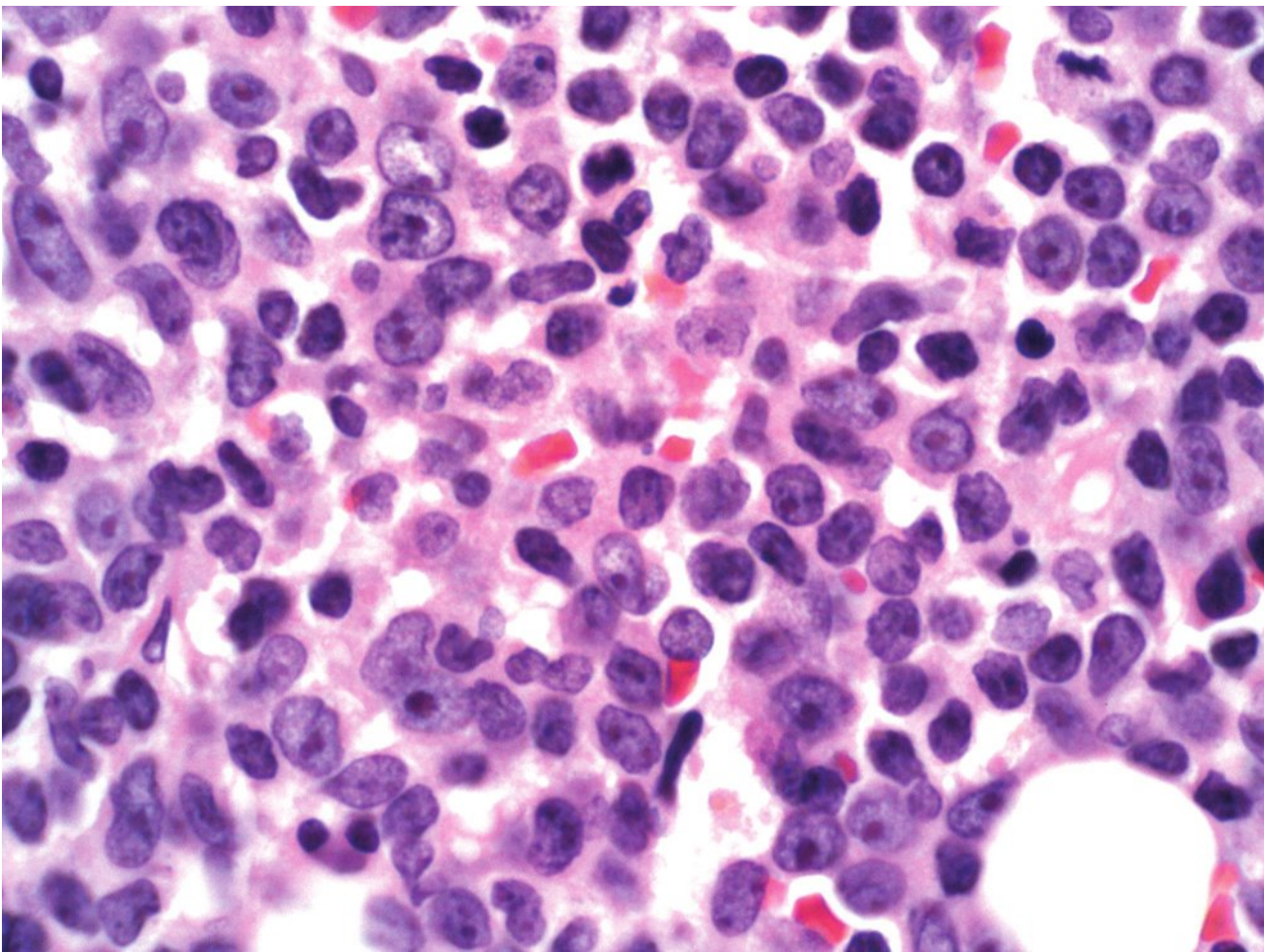


FIGURE 6.19.4 Bone marrow biopsy in a CLL case shows large lymphoid cell infiltration, consistent with diffuse large B-cell lymphoma transformation. Hematoxylin and eosin, 100× magnification.

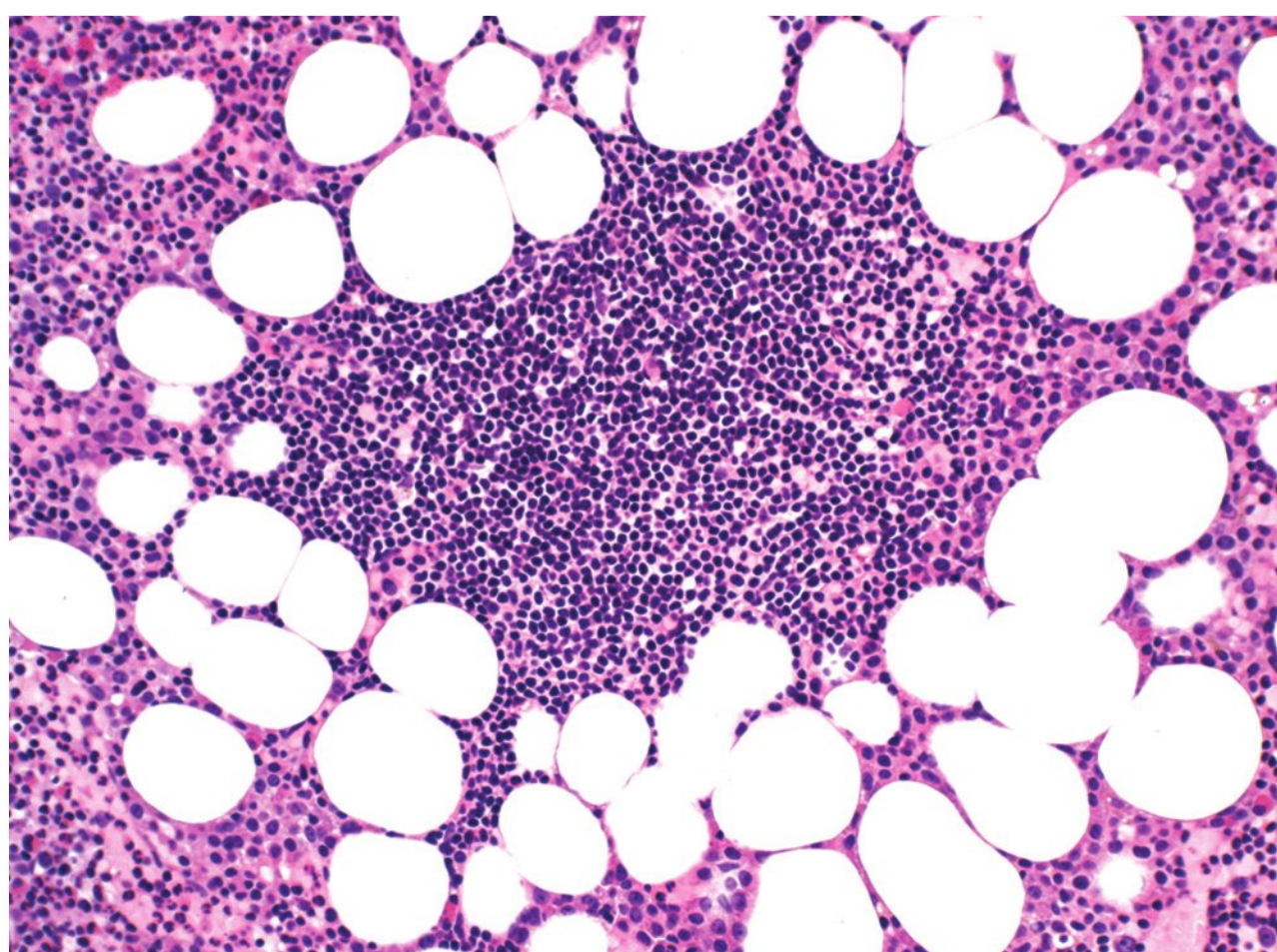


FIGURE 6.19.5 Bone marrow biopsy demonstrates a nodular lymphoid aggregate with infiltrating margin. Hematoxylin and eosin, 20× magnification.

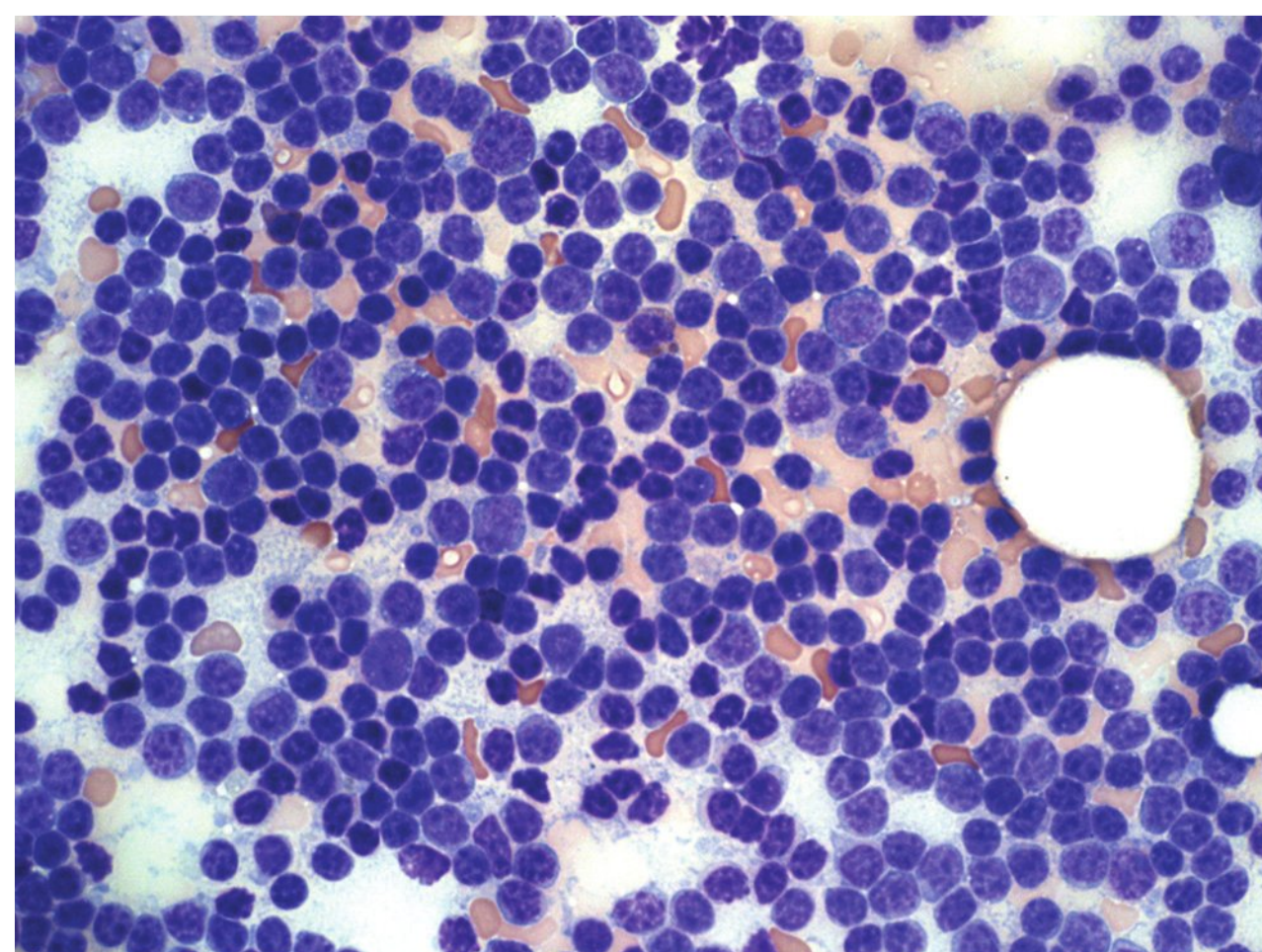


FIGURE 6.19.7 Bone marrow aspirate reveals diffuse lymphocytic infiltration. Wright-Giemsa, 40× magnification.

Recently, three more negative markers were added to the CLL panel for differential diagnosis. CD22, CD79b, and FMC-7 are either absent or weakly reactive in CLL cases (6,7,22). Thus, weak surface immunoglobulin, positive CD5 and CD23, negative or weakly positive CD22 or CD79b and FMC-7 are each counted as one point. When the tumor expresses more than three points, it is considered CLL; otherwise, it is likely to be other B-cell neoplasms.

When FMC-7 becomes positive in >20% of lymphoid cells in the peripheral blood, CLL/PLL should be considered. One study showed that high IgM fluorescence intensity, high FMC-7, and low CD23 expression in CLL cases were associated with a short survival (23). Those cases probably were in the process of prolymphocytoid transformation.

Other B-cell markers, such as CD21, CD24, and CD40, are also positive in CLL cells (3,12,20,24). HLA-DR is consistently demonstrated, but CD10 and CD79b have not

been detected on B-CLL cells (15). The terminal B-cell differentiation antigens, plasma cell antigen (PCA)-1 and CD38, are negative in CLL.

CLL cells may also express some myeloid antigens, including CD11b and CD11c, but not T-cell antigens other than CD5 (25). CLL cases expressing CD11c show features of both CLL and hairy cell leukemia (26).

In terms of adhesion molecules, CLL cells usually show low lymphocyte function-associated antigen (LFA)-1 (CD11/CD18) and CD54, intracellular adhesion molecule-1 (ICAM-1), but high Leu-8 (L-selectin) and CD44 (the lymphocyte homing receptor), whereas those of small lymphocytic lymphoma show high LFA-1 (24,27). The difference in the LFA-1 level is considered the mechanism that explains why CLL is disseminated, whereas small lymphocytic lymphoma stays in the lymph node.

Comparison between Flow Cytometry and Immunohistochemistry

The demonstration of CD5/CD19 (CD20) coexpression by flow cytometry is most important for the diagnosis of CLL, which cannot be accomplished by immunohistochemistry. The presence of CD5 and CD23 helps to distinguish CLL from many other lymphomas. The absence of CD10 excludes the leukemic phase of follicular lymphoma. However, about 10% to 15% of mantle cell lymphoma may express CD23. In those cases, immunohistochemical stain for cyclin D1 in the bone marrow biopsy is useful to distinguish CLL from mantle cell lymphoma. When solid organ is involved, immunohistochemistry is more helpful in differential diagnosis than flow cytometry.

The current case is typical for CLL in its natural history, morphology, and immunophenotype. This case, just like many of the CLL cases, was found incidentally during a routine physical examination. The patient was asymptomatic, with no lymphadenopathy, hepatosplenomegaly, anemia, or thrombocytopenia. However, the presence of >10,000/mL of lymphocyte in the peripheral blood and

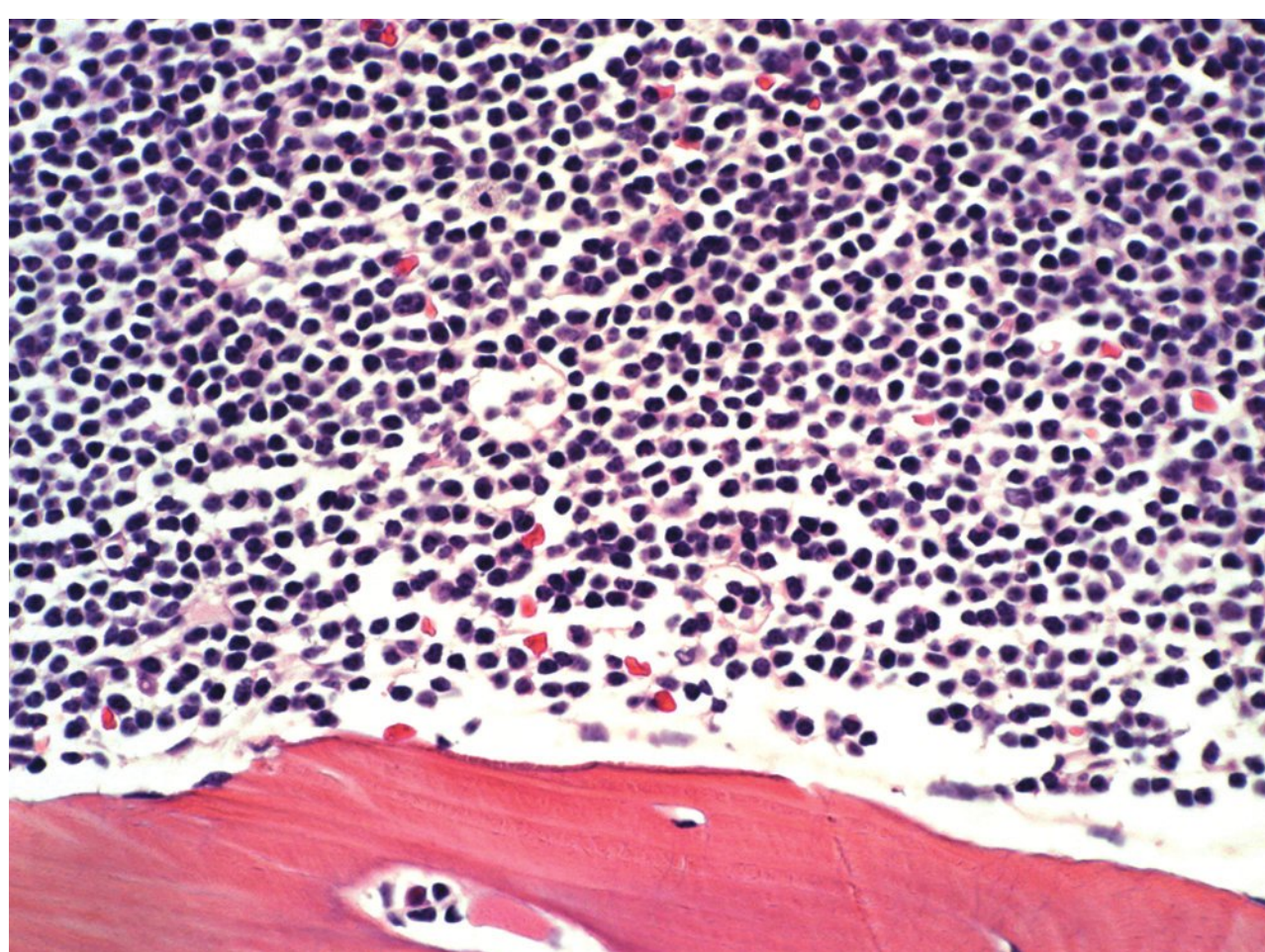


FIGURE 6.19.6 Bone marrow biopsy shows a diffuse infiltration pattern without residual fat vacuoles. Hematoxylin and eosin, 40× magnification.

>30% of lymphocytes in the bone marrow met the basic criteria of CLL. Flow cytometric analysis showed an immunophenotype of CD19/CD5+, CD20+, CD23+, dim monoclonal k pattern, and weak FMC-7. This finding further confirmed the diagnosis of CLL. Because the patient was asymptomatic, with no lymphadenopathy, hepatosplenomegaly, anemia, or thrombocytopenia, he was not treated despite the fact that the lymphocyte counts increased steadily over a 4-year period. It is expected that this patient may enjoy a normal life span until he has severe superimposed infection or transformation to a high-grade lymphoid neoplasm.

Molecular Genetics

Cytogenetic study is not necessary for the diagnosis of CLL, but an aberrant karyotype may help in differential diagnosis and in prediction of prognosis. With conventional karyotyping, chromosomal abnormalities can be demonstrated in 40% to 50% of CLL cases, but interphase fluorescence in situ hybridization (FISH) may disclose approximately 80% abnormalities (28). Using karyotyping, trisomy 12 was found to be the most common cytogenetic aberration, but with FISH, 13q- and 11q- are detected more frequently than trisomy 12 in CLL cases (28).

The frequently quoted frequencies of cytogenetic aberrations as demonstrated by FISH in CLL cases are as follows: del 13q14, 50%; del 11q22-q23, 20%; trisomy 12, 15%; del 6q21, 10%; and del 17p13, 5% to 10% (29–32).

The significance of del 13q14 is due to the fact that two microRNA clusters, miR-15a and miR-16-1, are located within this deleted region. MIRN15A and MIRN16-1 genes are present at chromosome 13q14.3 and are ubiquitously expressed, as noncoding RNAs (miR-15a and miR-16-1) with highest levels in normal CD5+ lymphocytes (33). In normal tissues, miR-15a and miR-16-1 are expressed in high levels, but in the majority of CLL patients MIRN15A and MIRN16-1 are down-regulated. Similar to del 13q14.3, downregulation of miR-15a and miR-16-1 is associated with good prognosis (33). A microRNA expression signature including miR-15a and miR-16-1 has been reported to distinguish between mutated and unmutated CLL and between ZAP-70-negative and ZAP-70-positive CLL cases (34).

Deletion of 11q is associated with distinct clinical manifestations, including young age, male gender, bulky lymphadenopathy, and poor prognosis (32). The fact that the ataxia telangiectasia mutated (ATM) gene is located within the minimum region of loss at 11q23 suggests alterations in DNA repair pathway may be involved in the pathogenesis of CLL (28,32,35).

The prognostic effect and the role in leukemogenesis of trisomy 12 are controversial (28,32,36), but it is frequently associated with Richter transformation (13,37). The role of del 6q in the pathogenesis of CLL is unknown.

Deletion 17p is associated with rapid progression of disease, poor response to therapy, and short survival (32). The deletion involves TP53 locus at 17p13 and TP53 mutation is associated with poor prognosis and refractory to conventional therapy even in the absence of del 17p (31,32). A higher percentage (42%) of TP53 mutation was found in CLL cases with Richter transformation than in those without (15%) (38). TP53 abnormality is also more

frequently found in CLL cases with >10% prolymphocytes (8 of 15 cases) than those with <10% prolymphocytes (3 of 17 cases) (39). In addition, CLL/PLL cases showed biallelic inactivation of TP53, whereas typical CLL cases affected only one allele (39). Another study also proposed that both TP53 and Rb tumor suppressor genes may play a role in clonal evolution of CLL cases (40). Current data show that Richter transformation is also associated with abnormalities in cell cycle regulation (e.g., loss of the cell cycle inhibitors CDKN1A and CDKN1B) and DNA repair (e.g., mutations and/or deletions of TP53, ATM, and CDKN2A and epigenetic silencing of MLH1) (13).

Chromosome translocations between the bcl-2 oncogene on 18q21 and one of the immunoglobulin genes are found in 1% to 4% of B-CLL cases (35). One of the immunoglobulin genes may involve the heavy-chain gene t(14;18), k light-chain gene t(2;18), or l light-chain gene t(18;22). The translocation of t(14;18) is more frequently seen in follicular lymphoma, whereas t(2;18) and t(18;22) are more frequently demonstrated in B-CLL. Despite the rarity of BCL-2 gene rearrangement in B-CLL, BCL-2 messenger RNA (mRNA) and protein expression are quite common. The mechanism of bcl-2 overexpression in CLL is due to BCL-2 promoter region DNA hypomethylation rather than gene rearrangement (41). Chromosome 11 may also translocate with chromosome 14 in CLL cases (42–45). However, this translocation is commonly seen in mantle cell lymphoma, which should be excluded before diagnosing CLL.

In terms of prognosis, the most important molecular genetic finding in recent years is the stratification of CLL cases into two groups on the basis of the mutational status of the variable region of the immunoglobulin heavy-chain gene (V_H gene). When the V_H gene shows >2% difference of nucleotide sequences from germline cells, it is considered mutated. Accordingly, B-cell neoplasms can be assigned into various developmental stages: pregerminal center stage with no somatic mutations, germinal center stage with mutations and ongoing mutational activities, and postgerminal center stage with a stable pattern of mutations (46).

It has been found that CLL cases have either unmutated or mutated V_H genes; the former has a poor prognosis, whereas the latter has a good prognosis (47,48). In comparison with normal B-cell differentiation, these two groups are assumed to be derived from naïve B cell and memory B cell, respectively (46). Gene expression profiling studies discovered that both mutated and unmutated groups share common expression levels of many genes, and this profile is distinct from that of normal B cells or other B-cell malignancies (49). However, there are small subsets of genes that are different between mutated and unmutated CLL cases. One of these genes is zeta-associated protein 70 (ZAP-70), which can be identified at RNA and protein levels (46,50).

ZAP-70 directly enhances the B-cell receptor transduction and IgM signaling in CLL cases, leading to increased tyrosine phosphorylation of key signal transduction proteins (51). As a result, the unmutated CLL cells are activated, receiving continued stimulation for division and proliferation (34,46). In contrast, the mutated CLL cells are in the anergic state with down-regulated B-cell receptor and reduced signal reception with resultant less aggressive behavior (46).

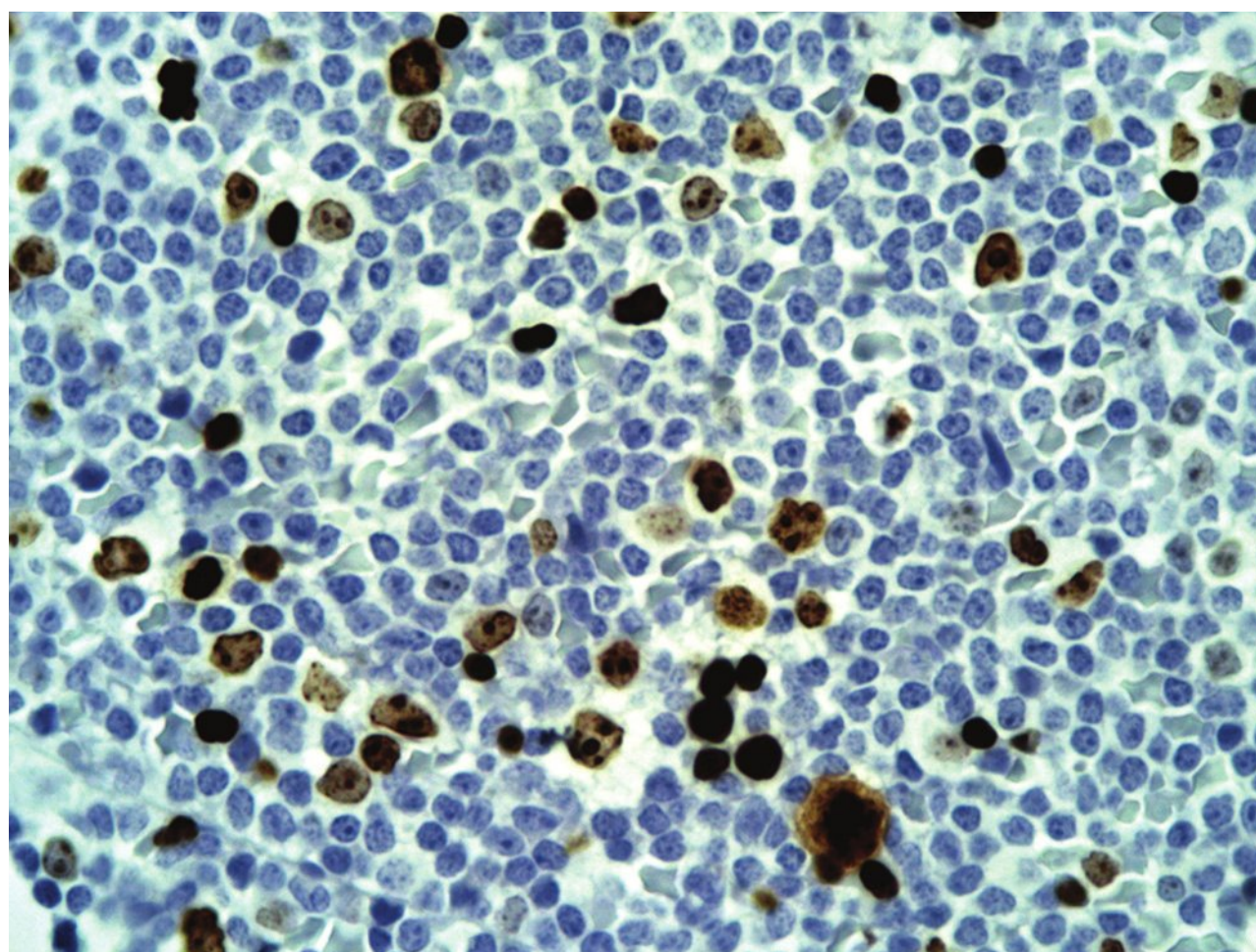


FIGURE 6.19.8 Bone marrow biopsy demonstrates Ki-67–positive large lymphocytes. The Ki-67 stain highlights the nucleoli in some cells. Immunoperoxidase, 60× magnification.

In the microenvironment, the interactions between CLL cells and the stromal cells, or nurse-like cells or interactions between CD38 and its natural ligand CD31 may rescue CLL cells from apoptosis (35,52). This phenomenon occurs preferentially in lymph-node pseudofollicles and bone marrow clusters, evidenced by the demonstration of Ki-67 on the leukemic cells in these sites (Fig. 6.19.8) (35).

As mentioned above, the interaction between CD38 and CD31 may inhibit apoptosis. When CD38 is upregulated and reaches its signaling threshold, it can also deliver proliferation signals (52). As a result, the expression of CD38 in CLL cells is usually associated with a poor prognosis. Early studies indicated the close relationship between CD38 and the unmutated status of CLL cells (47). However, subsequent studies found that these two parameters did not always correlate (34). Nevertheless, many studies confirm CD38 as an independent inverse prognosticator when a high percentage (>30%) and an elevated level of antibody-binding capacity (>25%) of CD38 are demonstrated (49). A recent study has found that high expression of CD25

TABLE 6.19.2

Salient Features for Laboratory Diagnosis of B-CLL

1. Peripheral lymphocytosis with an absolute lymphocyte count of >5,000/mL
2. Bone marrow lymphocytosis of >30% of the total population
3. Diagnostic score points: dim surface immunoglobulin, positive CD5, positive CD23, negative FMC-7, weak CD22 or CD79b (1 point assigned to each marker, >3 points is suggestive of CLL)
4. Monoclonal immunophenotype
5. Positive immunoglobulin gene rearrangement
6. Differentiation markers: CD10 (follicular lymphoma), FMC-7 (PLL), CD103/CD123 (hairy cell leukemia), cyclin D1 (mantle cell lymphoma)
7. Cytogenetics: Usually normal; 13q–, 11q–, +12, 6q–, and 17p– are relatively common.

is also associated with unmutated V_H gene and confers a poor prognosis (53).

Another finding connecting unmutated V_H gene with poor prognosis is that CLL cells from this group of patients have a shorter telomere length and a higher telomerase activity than those from patients with mutated status (22).

The salient features for laboratory diagnosis of CLL are summarized in Table 6.19.2.

Clinical Manifestations

CLL patients are usually older than 50 years with a male-to-female ratio of 2:1. The organ or tissue involvement depends on the stage of the disease. The Rai staging system is based on the presence or absence of bone marrow involvement, lymphadenopathy, hepatomegaly, splenomegaly, anemia, and thrombocytopenia to divide CLL into five stages (Table 6.19.3) (3). In terms of prognosis, the five stages of the Rai system can be condensed into three stages: stage 0

TABLE 6.19.3

Rai Staging System for CLL

	Stage 0	Stage I	Stage II	Stage III	Stage IV
Peripheral and marrow lymphocytosis	+	+	+	+	+
Lymphadenopathy	-	+	±	±	±
Hepatomegaly/splenomegaly	-	-	+	±	±
Anemia (Hb < 11g/dL)	-	-	-	+	±
Thrombocytopenia (platelets < 100,000/ μ L)	-	-	-	-	+



predicts good prognosis; stages I and II, intermediate prognosis; and stages III and IV, poor prognosis (54). Binet et al. (55) defined a new staging system, similar to the Rai condensed staging system, with the major differences in counting five involved areas (cervical, axillary, and inguinal lymph nodes; spleen; and liver) and in lowering the cutoff point of the hemoglobin level to 10 g/dL (Table 6.19.4).

With the improvement of diagnostic techniques, many CLL cases are now diagnosed at an early stage. Some patients may survive for many years without therapy, and others may progress rapidly. With the advances in treatments such as purine analogs, monoclonal antibodies, and bone marrow transplant, CLL patients can achieve high response rates, even with molecular remissions (27). Therefore, it has become obvious that patients should be treated before they meet the therapeutic guideline recommended by the National Cancer Institute Working Group (including the development of B symptoms, worsening anemia and/or thrombocytopenia, autoimmune cytopenias, progressive splenomegaly, progressive lymphadenopathy, and lymphocyte doubling time of 6 months) (56).

The recent stratification of CLL patients into two groups based on V_H gene mutational status provides a reliable indicator for therapeutic targeting: those patients with unmutated V_H gene should be treated, and those with mutated status should be watched and waited upon (35,47,48). The medium survival was 8 to 9 years for patients with unmutated status but beyond 24 years for those with mutated status (47,48).

As mentioned before, the unmutated CLL cells usually express ZAP-70 and CD38, which also substantiate the prediction of poor prognosis. Cytogenetic abnormalities are also reliable in defining high- and low-risk groups (1,22,35,50). For instance, del 13q confers a better survival, whereas del 11q and del 17p predict an ominous outcome. The lymphocyte doubling time of <6 months (56) and a diffuse infiltration pattern in the bone marrow (16) are also bad signs. Serum markers that are useful for monitoring patients include lactate dehydrogenase, beta-2 microglobulin, thymidine kinase, and soluble CD23 levels (1,22,50). The prognostic factors that help stratify the high-risk and low-risk groups of CLL are summarized in Table 6.19.5.

TABLE 6.19.4

Binet Staging System for CLL

Stage	Areas involved*	Hemoglobin (g/ dL)	Platelets ($\times 10^9$ / L)
A	<3	≥ 10	≥ 100
B	≥ 3	≥ 10	≥ 100
C	Variable	<10	<100

*The areas include cervical, axillary, and inguinal lymph nodes as well as spleen and liver.

TABLE 6.19.5

Prognostic Factors in CLL

Parameter	Low risk	High risk
Clinical stage	Rai 0, I; Binet A	Rai II, III, IV; Binet B or C
Lymphocyte morphology	Typical	Atypical
Bone marrow pattern	Nondiffuse	Diffuse
Lymphocyte doubling time	>12 mo	<12 mo
Serum markers*	Normal	Elevated
CD38 expression	<20%–30%	>20%–30%
ZAP-70 expression	<20%	>20%
Genetic abnormalities	Normal, del 13q	del 11q, del 17p
V_H gene status	Mutated	Unmutated

*Serum markers include lactate dehydrogenase, beta-2 microglobulin, thymidine kinase, and soluble CD23.

ZAP-70, zeta-associated protein 70; V_H gene, immunoglobulin variable heavy-chain gene.

Oscier D, Fegan C, Hillmen P, et al. Guidelines on the diagnosis and management of chronic lymphocytic leukemia. *Br Med J*. 2004;125:294–317; Gentile M, Mauro FR, Guarini A, et al. New development in the diagnosis, prognosis and treatment of chronic lymphocytic leukemia. *Curr Opin Oncol*. 2005;17:597–604; and Shanafelt TD, Geyer SM, Kay NE. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood*. 2004;103:1202–1210.

REFERENCES

- Oscier D, Fegan C, Hillmen P, et al. Guidelines on the diagnosis and management of chronic lymphocytic leukemia. *Br Med J*. 2004;125:294–317.
- Morton LM, Wang SS, Devesa SS, et al. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. *Blood*. 2006;107:265–276.
- Dighiero G, Travade P, Chevade P, et al. B-cell chronic lymphocytic leukemia: present status and future directions. *Blood*. 1991;78:1901–1914.
- Linnet MS, Schubauer-Berigan MK, Weisenburger DD, et al. Chronic lymphocytic leukaemia: an overview of aetiology in light of recent developments in classification and pathogenesis. *Br J Haematol*. 2007;139:672–686.
- Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. *Blood*. 1975;46:219–234.
- Hallek M, cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood*. 2008;111:5446–5456.
- Müller-Hermelink HK, Monteserrat E, Catovsky D, et al. Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:180–184.
- Nowakowski GS, Hoyer JD, Shanafelt TD, et al. Using smudge cells on routine blood smears to predict clinical outcome in chronic lymphocytic leukemia: a universally available prognostic test. *Mayo Clin Proc*. 2007;82:449–453.
- Melo JV, Robinson DSF, Catovsky D. The differential diagnosis between chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders: morphological and immunological studies. In: Pooliack A, Catovsky D, eds. *Chronic Lymphocytic Leukemia*. Chur, Switzerland: Harwood Academic Publishers; 1988:85–103.
- Gale RP, Foon KA. Biology of chronic lymphocytic leukemia. *Semin Hematol*. 1987;24:209–229.
- Stark A, Limbert H, Robert B, et al. Prolymphocytoid transformation of chronic lymphocytic leukemia: a clinical and immunological study of 22 cases. *Leuk Res*. 1986;10:1225–1232.
- Foon K, Gale R. Clinical transformation of chronic lymphocytic leukemia. *Nouv Rev Fr Hematol*. 1988;30:385–388.
- Omori CE, Omori AE. Richter syndrome: a review of clinical, ocular, neurological and other manifestations. *Br J Haematol*. 2008;142:709–716.
- Foucar K. Chronic lymphoid leukemias and lymphoproliferative disorders. *Mod Pathol*. 1999;12:141–150.
- Foucar K. B-cell chronic lymphocytic leukemia and prolymphocytic leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1505–1529.
- Rozman C, Monteserrat E, Rodriguez-Fernandez JM, et al. Bone marrow histologic pattern—the best single prognostic parameter in chronic lymphocytic leukemia. A multivariate survival analysis of 329 cases. *Blood*. 1984;64:642–648.
- Binet JL, Calignris-Cappio F, Catovsky D, et al. Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. *Blood*. 2006;107:859–861.
- Swerdlow SH. Small B-cell lymphomas of the lymph nodes and spleen: practical insight to diagnosis and pathogenesis. *Mod Pathol*. 1999;12:125–140.
- Johnstone AP. Chronic lymphocytic leukemia and its relationship to normal B-lymphopoiesis. *Immunol Today*. 1982;3:342–348.
- Tefferi A, Phylly RL. A clinical update on chronic lymphocytic leukemia. 1. Diagnosis and prognosis. *Mayo Clin Proc*. 1992;67:349–353.
- Kilo MN, Dorfman DM. The utility of flow cytometric immunophenotypic analysis in the distinction of small lymphocytic lymphoma/chronic lymphocytic leukemia from mantle cell lymphoma. *Am J Clin Pathol*. 1996;105:451–457.
- Gentile M, Mauro FR, Guarini A, et al. New development in the diagnosis, prognosis and treatment of chronic lymphocytic leukemia. *Curr Opin Oncol*. 2005;17:597–604.
- Geisler CH, Larsen JH, Hensen NE, et al. Prognostic importance of flow cytometric immunophenotyping of 540 consecutive patients with B-cell chronic lymphocytic leukemia. *Blood*. 1991;78:1795–1805.
- Freedman AS. Cell surface antigens in leukemias and lymphomas. *Cancer Invest*. 1996;14:252–276.
- Morabito F, Prasthoder EF, Dunlap NE, et al. Expression of myelomonocytic antigens on chronic lymphocytic leukemia B-cell correlation with their ability to produce interleukin 1. *Blood*. 1987;70:1750–1757.
- Hanson CA, Gribbin RE, Schnitzer B, et al. CD11c (Leu-M5) expression characterizes a B-cell chronic lymphoproliferative disorder with features of both chronic lymphocytic leukemia and hairy cell leukemia. *Blood*. 1990;76:2360–2367.
- Angelopoulou MK, Kontopidou FN, Pangalis GA. Adhesion molecules in B-chronic lymphoproliferative disorders. *Semin Hematol*. 1999;36:178–197.
- Dohner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343:1910–1916.
- Stilgenbauer S, Dohner H, Lichter P. Genomic aberrations in B-cell chronic lymphocytic leukemia. In: Cheson B, ed. *Chronic Lymphocytic Leukemia*. 2nd ed. New York: Marcel Dekker; 1993:353–376.
- Caporaso N, Goldin L, Plass C, et al. Chronic lymphocytic leukaemia genetics overview. *Br J Haematol*. 2007;139:630–634.
- Monteserrat E, Moreno C. Genetic lesions in chronic lymphocytic leukemia: clinical implications. *Curr Opin Oncol*. 2009;21:609–614.
- Butler T, Gribben JG. Biologic and clinical significance of molecular profiling in chronic lymphocytic leukemia. *Blood Rev*. 2010;24:135–141.
- Nicoloso MS, Kipps TJ, Croce CM, et al. MicroRNAs in the pathogeny of chronic lymphocytic leukaemia. *Br J Haematol*. 2007;139:709–716.
- Calin GA, Ferracin M, Cimmino A, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*. 2005;353:1793–1801.
- Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. *N Engl J Med*. 2005;352:804–815.
- Reed JC. Molecular biology of chronic lymphocytic leukemia. *Semin Oncol*. 1998;25:11–18.
- Garcia-Marco JA, Caldas C, Price CM, et al. Frequent somatic deletion of the 13q12.3 locus encompassing BCRA2 in chronic lymphocytic leukemia. *Blood*. 1996;88:1568–1575.
- Gaidano G, Ballerini P, Gong JZ, et al. p53 mutations in human lymphoid malignancies: association with Burkitt lymphoma and chronic lymphocytic leukemia. *Proc Natl Acad Sci U S A*. 1991;88:5413–5417.
- Lens D, Dyer MJ, Garcia-Marco JM, et al. p53 abnormalities in CLL are associated with excess of prolymphocytes and poor prognosis. *Br J Haematol*. 1997;99:848–857.

40. Kay NE, Ranheim EA, Peterson LC. Tumor suppressor genes and clonal evolution in B-CLL. *Leuk Lymphoma*. 1995;18:41–49.
41. Bannerji R, Byrd JC. Update on the biology of chronic lymphocytic leukemia. *Curr Opin Oncol*. 2000;12:22–29.
42. Cuneo A, Balboni M, Piva N, et al. Atypical chronic lymphocytic leukemia with t(11;14)(q13;q32): karyotype evolution and prolymphocytic transformation. *Br J Haematol*. 1995;90:409–416.
43. Brizard F, Dreyfu B, Buihor F, et al. 11q13 rearrangement in B cell chronic lymphocytic leukemia. *Leuk Lymphoma*. 1997;25:539–543.
44. Coignet LJ, Schuurin E, Kibbelaar RE, et al. Detection of 11q13 rearrangements in hematologic neoplasias by double-color flow fluorescence in situ hybridization. *Blood*. 1996;87:1512–1519.
45. Takashima T, Itoh M, Ueda Y, et al. Detection of 14q32.33 translocation and t(11;14) in interphase nuclei of chronic B-cell leukemias/lymphomas by in situ hybridization. *Int J Cancer*. 1997;72:31–38.
46. Stevenson FK, Caligaris-Cappio F. Chronic lymphocytic leukemia: revelations from the B-cell receptor. *Blood*. 2004;103:4389–4395.
47. Damle RN, Wasil T, Fais F, et al. IgV gene status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94:1840–1847.
48. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated IgVH genes associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848–1854.
49. Kipps TJ. Immunobiology of chronic lymphocytic leukemia. *Curr Opin Hematol*. 2003;10:312–318.
50. Shanafelt TD, Geyer SM, Kay NE. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. *Blood*. 2004;103:1202–1210.
51. Chen L, Apgar J, Huynh L, et al. ZAP-70 directly enhances IgM signaling in chronic lymphocytic leukemia. *Blood*. 2005;105:2036–2041.
52. Deaglio S, Capobianco A, Bergui L, et al. CD38 is a signaling molecule in B-cell chronic lymphocytic leukemia cells. *Blood*. 2003;102:2146–2155.
53. Sulda ML, Kuss BJ, Hall RK, et al. The clinical utility of molecular and flow cytometric markers in CLL. *Int J Med*. 2010; Epub ahead of print.
54. Rai KR. A critical analysis of staging in CLL. In: Gale RP, Rai KR, eds. *Chronic Lymphocytic Leukemia: Recent Progress and Future Directions*. New York: Alan R. Liss; 1987: 253–264.
55. Binet JL, Auquier A, Dighiero G, et al. A new prognostic classification of chronic lymphocytic leukemia: a retrospective multicentric study from the Giemsa group. *J Clin Oncol*. 1987;5:398–401.
56. Cheson BD, Bennett JM, Grever M, et al. National Cancer Institute sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*. 1996;87:4990–4997.

CASE 20

Chronic Lymphocytic Leukemia of T-Cell Lineage

CASE HISTORY

A 77-year-old man presented with abdominal pain and was admitted to the hospital on the diagnosis of appendicitis. The patient denied having fever, night sweats, weight loss, or bruising. On admission, the patient had a total leukocyte count of 34,000/mL with absolute lymphocytosis (19,300/mL) and neutrophilia (12,900/mL). After surgery, lymphocytosis persisted with an absolute lymphocyte count of 13,000/mL. Flow cytometric analysis revealed that the lymphocytes were predominantly CD4+ helper T cells, consistent with T-cell leukemia. Physical examination showed no lymphadenopathy but mild splenomegaly. The patient was not treated for leukemia, but was doing well after discharge.

Four months later, the patient presented with red cutaneous papules on the shins, which were considered either leukocytoclastic vasculitis or leukemic involvement of the skin. A skin biopsy demonstrated small mature-looking lymphocyte infiltration of the dermis, consistent with leukemic infiltration (Fig. 6.20.1). The leukocyte count had continued to rise and reached the level of 57,400/mL with

an absolute lymphocyte count of 51,000/mL (Fig. 6.20.2). Bone marrow examination showed 43.5% lymphocytes in the aspirate (Fig. 6.20.3) and large lymphoid aggregates in the core biopsy. Flow cytometric analysis of the bone marrow revealed a predominantly CD4-positive population similar to that seen in the peripheral blood analysis. During the second admission, splenomegaly was detected, the clinical course became rapidly progressive, and the patient failed to respond to chemotherapy. He was finally discharged to a hospice.

IMMUNOHISTOCHEMISTRY

Immunohistochemical stains of the bone marrow core biopsy showed that the lymphoid aggregates were positive for T-cell stains (CD3 and CD45RO) (Fig. 6.20.4), but negative for B-cell stain (CD 20). Immunohistochemical stains of the skin biopsy revealed that the leukemic cells were positive for CD3 and CD4 (Fig. 6.20.5) and negative for CD8.

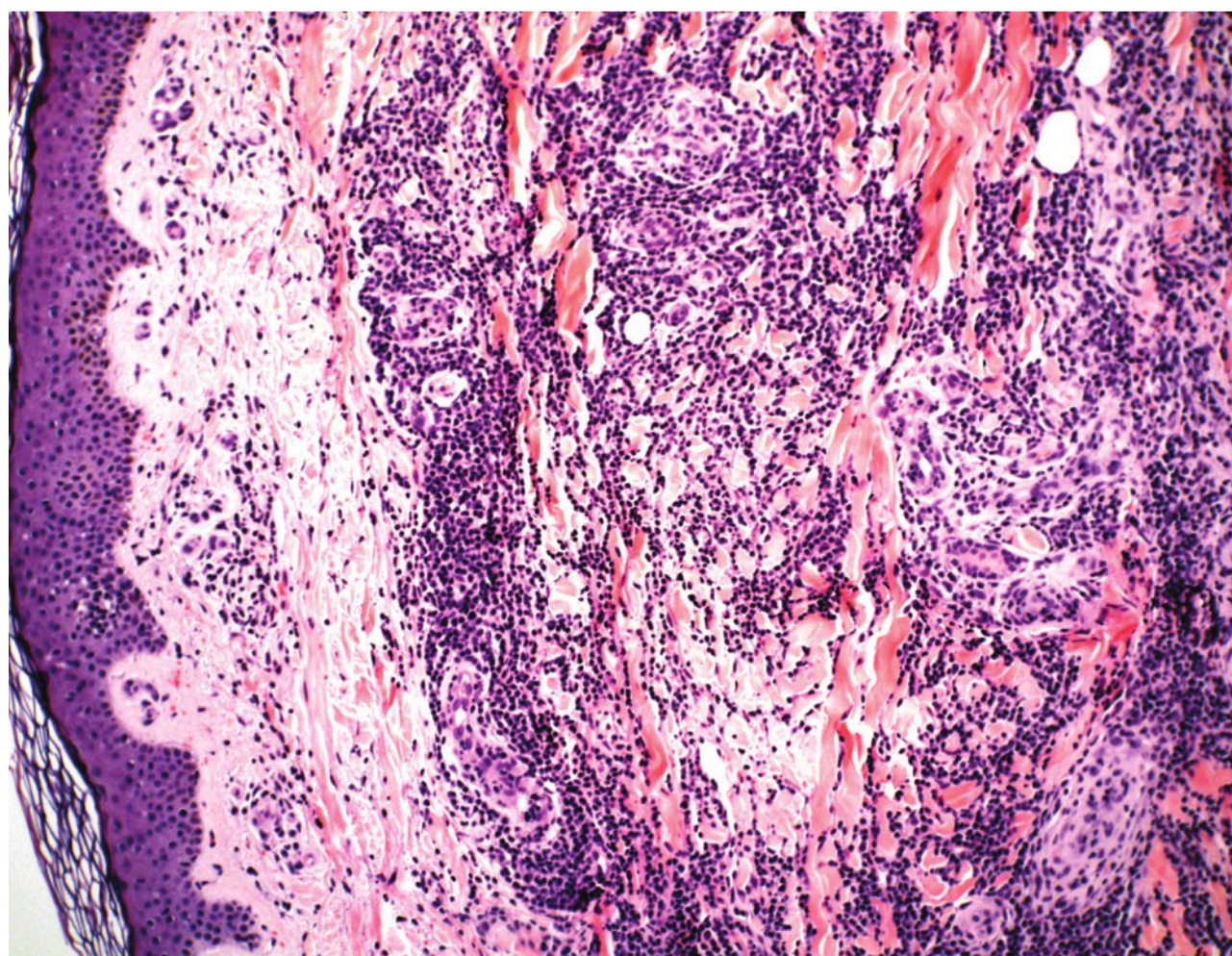


FIGURE 6.20.1 A skin biopsy shows perivascular and periadnexal infiltration by small leukemic cells in the dermis. There is no epidermal involvement. Hematoxylin and eosin, 10× magnification.

FLOW CYTOMETRY

Flow cytometric analysis of the peripheral blood: T-cell markers: CD2 98%, CD3 97%, CD3/CD4 96%, CD3/CD8 1%, CD5 98%, CD7 99%. B-cell markers: CD19 1%, CD20 1%, CD23 3%, CD19/k 1%, CD19/l 0%, CD10 0%. Activation antigen: CD25 6% (Fig. 6.20.6).

Flow cytometric analysis of the bone marrow: T-cell markers: CD2 95%, CD3 91%, CD3/CD4 86%, CD3/CD8 6%, CD5 95%, CD7 98%, TdT 0%. B-cell markers: CD19 3%, CD23 9%. Natural killer (NK)-cell markers: CD16 3%, CD56 4%, CD57 2%.

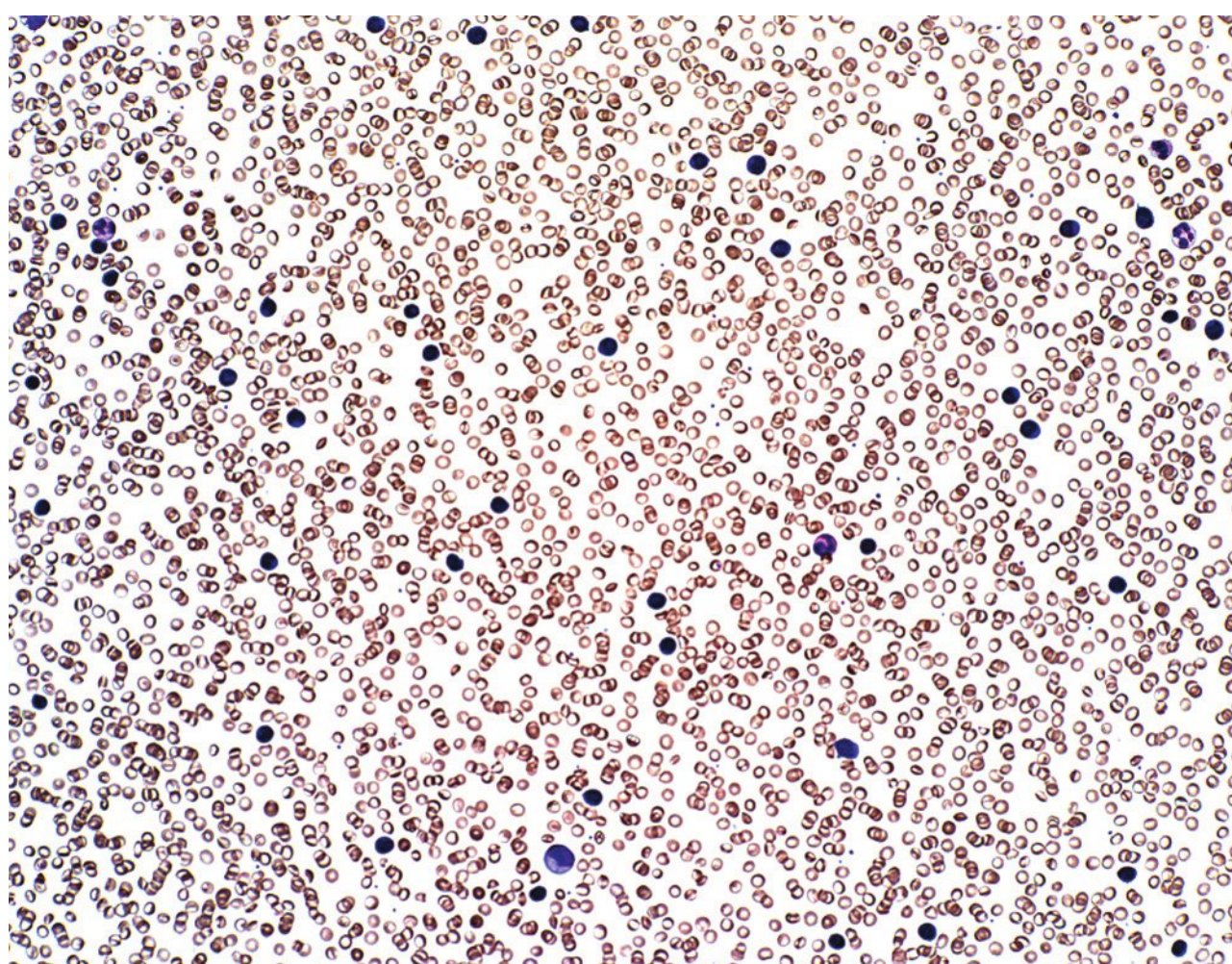


FIGURE 6.20.2 A peripheral blood smear shows numerous small, mature-looking lymphocytes, indistinguishable from those seen in B-cell CLL. Wright–Giemsa, 20× magnification.

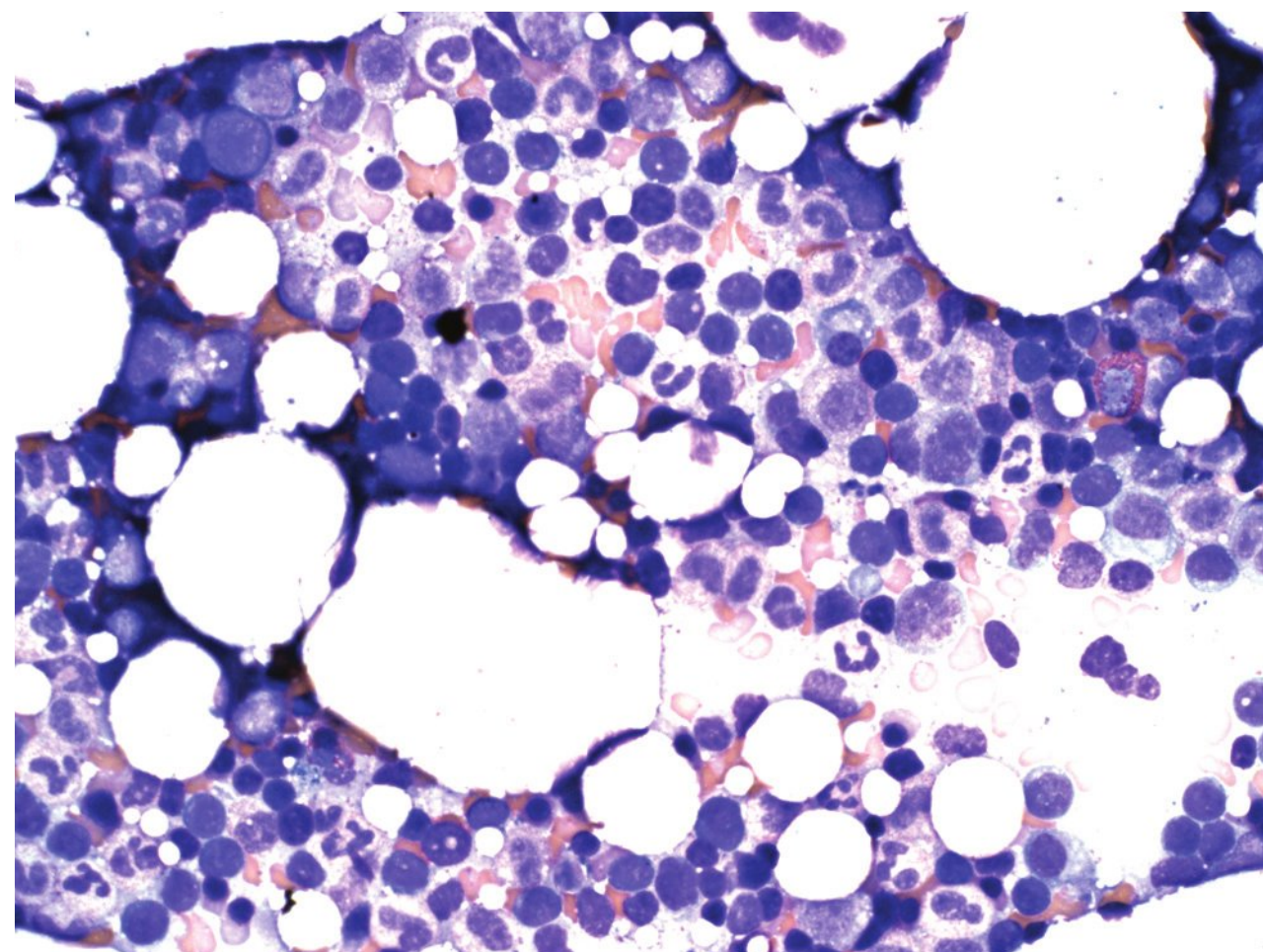


FIGURE 6.20.3 A bone marrow aspirate reveals a high percentage of small lymphocytes. Wright–Giemsa, 40× magnification.

MOLECULAR GENETICS

T-cell receptor g-chain gene rearrangement analysis of the peripheral blood specimen obtained from the first admission using polymerase chain reaction showed a clonal T-cell population. Cytogenetic study of the bone marrow obtained from the second admission revealed a normal male karyotype of 36XY.

DISCUSSION

Most cases of chronic lymphocytic leukemia (CLL) are of B-cell lineage. The frequency of the T-cell type of CLL (T-CLL) is only about 1% to 2% of the total of CLL cases (1).

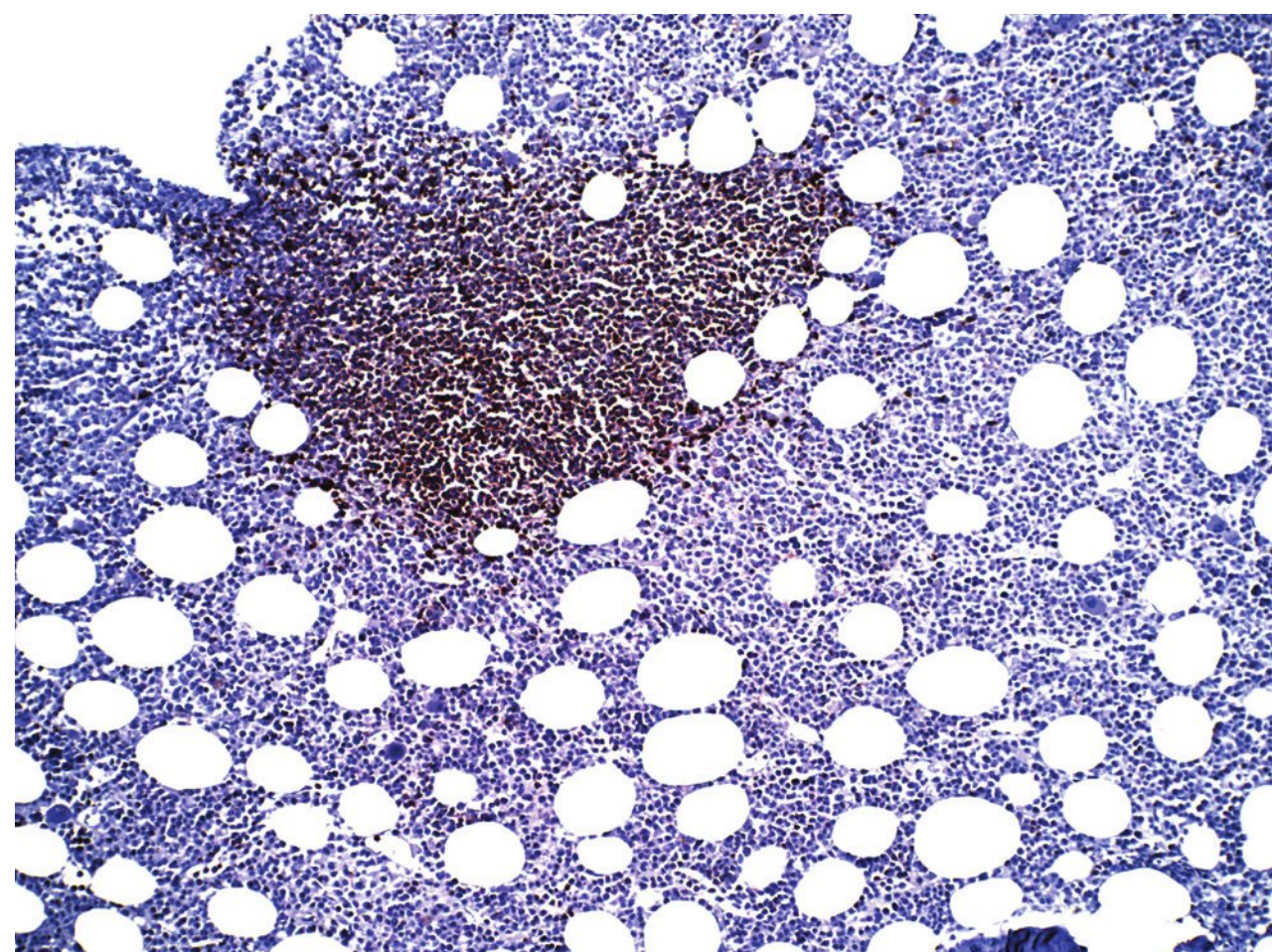


FIGURE 6.20.4 A bone marrow core biopsy shows a large lymphoid aggregate stained positive for CD3. Myeloperoxidase, 10× magnification.

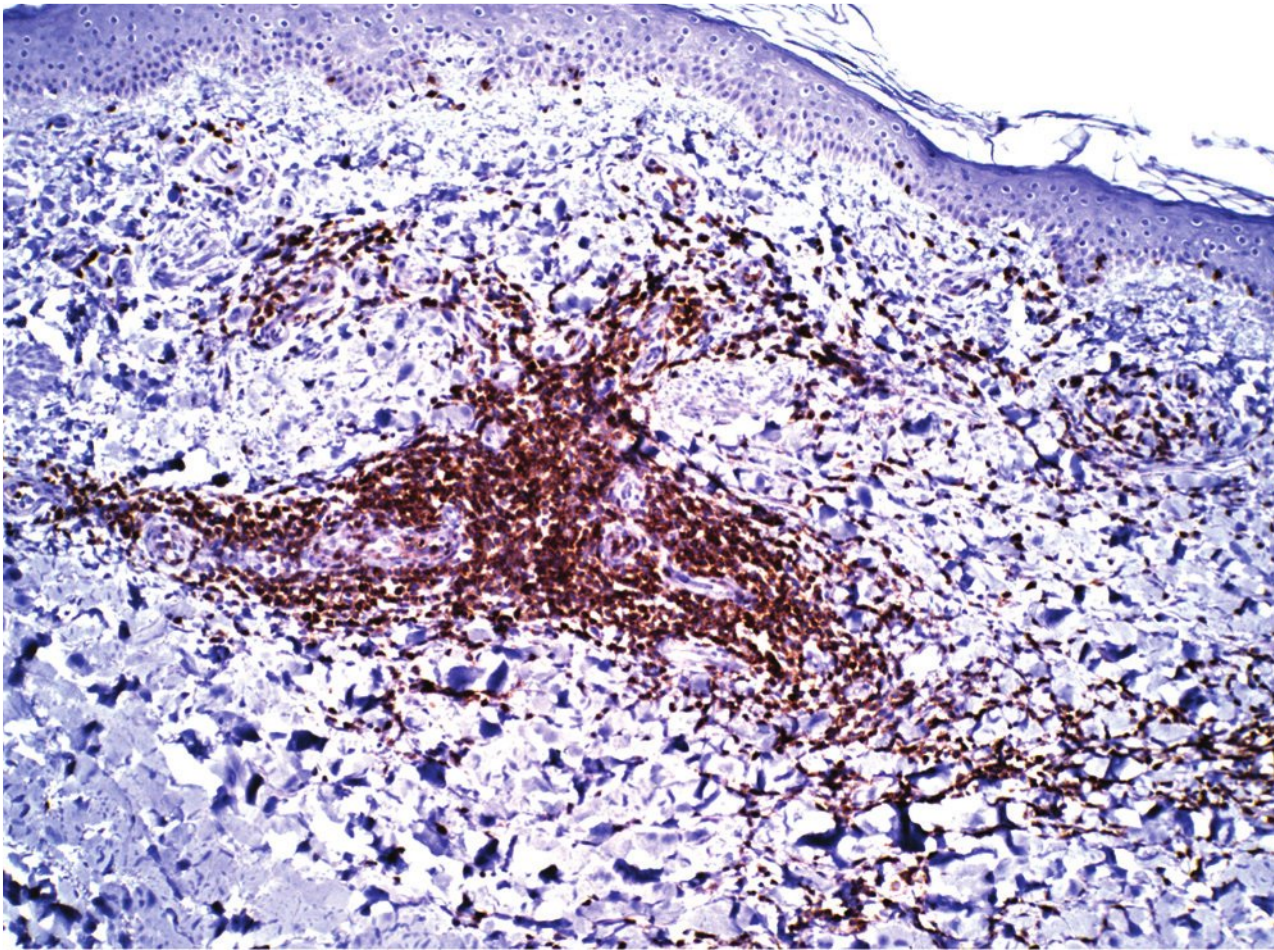


FIGURE 6.20.5 A skin biopsy reveals a leukemic infiltrate that stains for CD4. Immunoperoxidase, 10× magnification.

However, the incidence of T-CLL in Japan is proportionally higher, and the general incidence of all CLL cases is much lower than in the Western countries (2). In a survey of 134 CLL patients in Japan, the percentages of B-CLL and T-CLL are 78% and 18%, respectively (2).

In the early literature, T-CLL was classified into three types: the knobby type, the azurophilic type, and the adult T-cell leukemia/lymphoma (ATCL) (3,4). The azurophilic type is now included in the large granular lymphoproliferative disorders (LGLDs) (5). The ATCL is also separated from T-CLL (6). The knobby type was so called because of the frequent presence of nuclear protrusions in the tumor cells and still remains in the T-CLL category.

However, because cases of T-CLL usually have a high leukocyte count and an aggressive clinical course and, in some cases, share karyotypes similar to those of T-cell prolymphocytic leukemia (T-PLL), Matutes and Catovsky (7,8) advocated the inclusion of T-CLL into the T-PLL category and called it the small-cell variant of T-PLL. These authors claimed that, although nucleoli are not demonstrated in those T-CLL cells at light microscopic level, they should be visible under electron microscopy. This concept was supported by a few authors (9). The entity of T-CLL is retained in the Revised European-American Lymphoma Classification (10), but it is deleted from the World Health Organization classification (11,12).

Nevertheless, many authors still consider T-CLL a separate entity because, morphologically, T-CLL cells are indistinguishable from those of B-CLL and the classification should not be based on the results of electron microscopy and cytogenetics (4,13–16). Indeed, many cases of T-CLL

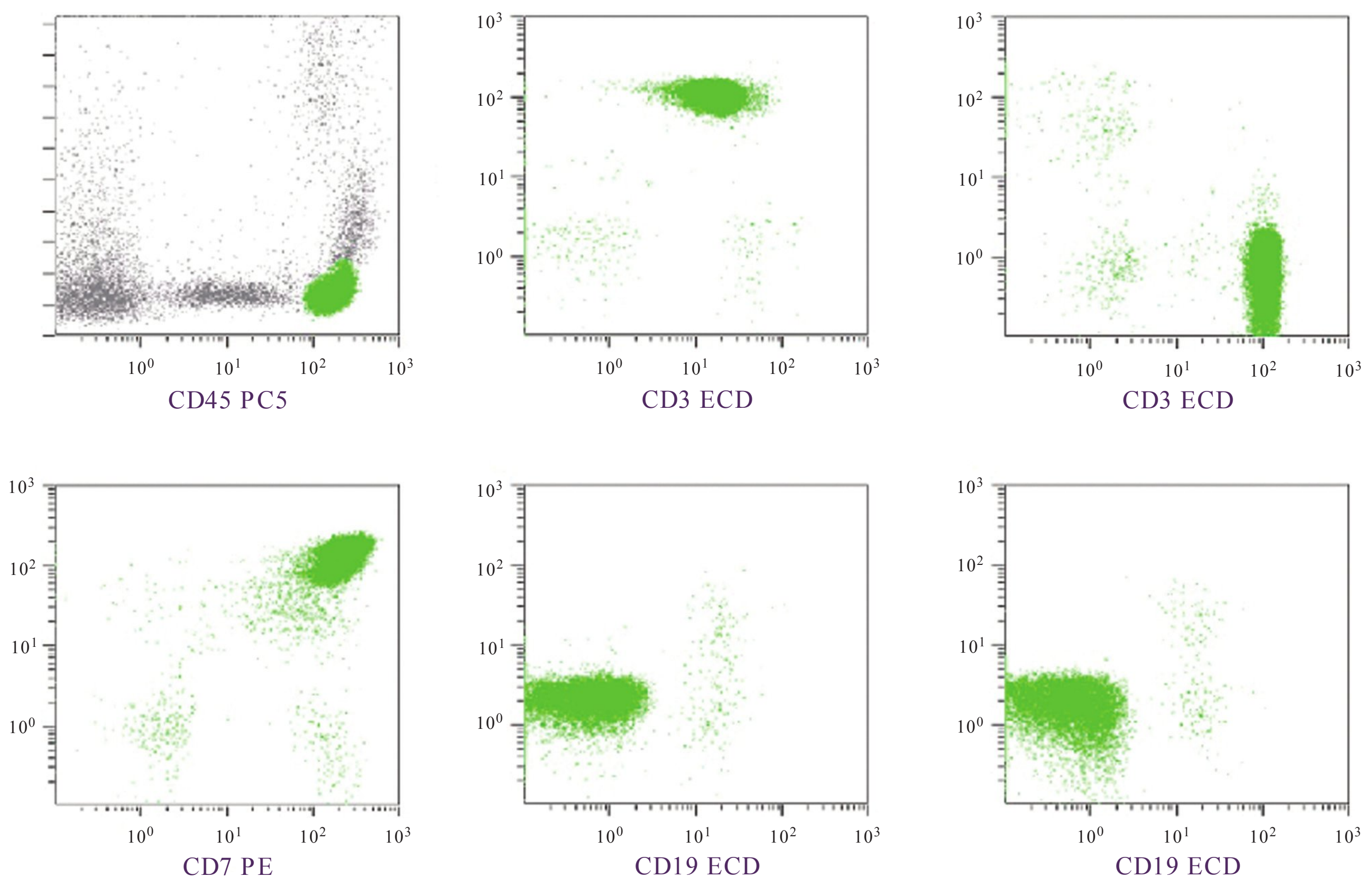


FIGURE 6.20.6 Flow cytometric histograms, with side-scatter versus CD45 gating, show positive reactions with CD3, CD4, CD5, and CD7 but negative reactions with CD8, CD19, and k and l stains. PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; ECD, phycoerythrin–Texas red.

do not have cytogenetic abnormalities that are similar to T-PLL, and splenomegaly, if present, is not as prominent as it is in cases of T-PLL.

In the current case, the tumor cell morphology is indistinguishable from that of B-CLL and the karyotype is not consistent with T-PLL. Although the aggressive clinical course is similar to that seen in T-PLL, it is not justifiable to label it as PLL on the basis of clinical presentation alone. The absence of azurophilic granules in the cytoplasm of the tumor cells and the lack of reactions to CD16, CD56, and CD57 are not consistent with LGLD. The cytology and the histologic pattern in the skin as well as the absence of CD25 reaction are not supportive of the diagnosis of ATCL. Therefore, it is reasonable to consider this case as T-CLL.

Morphology

Although early cases of T-CLL were described as knobby type for the presence of nuclear protrusion, most cases of T-CLL assume a morphology indistinguishable from B-CLL (4,13–16). In other words, they appear as small, mature-looking lymphocytes. In the study by Hoyer et al. (14), the median presenting lymphocyte count was 36,000/ μ L. The absence of cytoplasmic granules distinguishes T-CLL cells from large granular lymphocytes. The absence of

prominent nucleoli and the high nuclear/cytoplasmic ratio distinguish them from prolymphocytes.

Sézary syndrome is the most common T-cell leukemia and should be distinguished from T-CLL (17,18). The typical Sézary cells usually show a cerebriform nucleus and appear in the peripheral blood in the late stage of mycosis fungoides. When Sézary-like cells are present in the peripheral blood without skin involvement, it is called Sézary leukemia (19). This entity is probably a variant of T-PLL.

Another differential diagnosis for T-CLL is the ATCL, the tumor cells of which are characterized by the presence of polylobulated nuclei and are generally described as flower cells. These characteristic tumor cells, however, are present only in small numbers, often accounting for only about 5% of peripheral leukocytes (20). Furthermore, the tumor cells may show a cerebriform nucleus, indistinguishable from those of Sézary syndrome.

In the bone marrow, the infiltration pattern of T-CLL is usually interstitial, and the degree of involvement ranges from 15% to 90% (14). This is in contrast to the B-CLL cases, in which bone marrow involvement is usually prominent even at the early stage of the disease (1).

The differences among T-CLL, T-PLL, LGLD, ATCL, and Sézary syndrome are summarized in Table 6.20.1 (21).

TABLE 6.20.1

Differential Diagnosis of Peripheral T-Cell Leukemia

	T-CLL	ATCL	LGLD	T-PLL	Sézary syndrome
Cell morphology	Small mature lymphocytes	Lymphocytes with polylobulated nuclei	Large granular lymphocytes	Prolymphocytes	Lymphocytes with cerebriform nuclei
Lymphocytosis	Moderate to marked	Moderate	Relative (neutropenia)	Marked	Moderate
Splenomegaly	Common	Variable	Common	Common	Rare
Lymphadenopathy	Common	Common	No	Variable	Common
Skin infiltration	Common	Common	No	Variable	Common
Pan-T antigens*	All positive	All positive except CD7	CD2+, others $\pm^{\dagger}$	All positive	All positive except CD7
T-cell subset	CD4+ CD8–	CD4+ CD8–	CD4– CD8+	Variable	CD4+, CD8–
CD25 (IL-2R)	$\pm$	+	–	$\pm$	$\pm$
Activation antigens [‡]	$\pm$	+	$\pm$	$\pm$	$\pm$
TdT and CD1a	–	–	–	–	–
NK-cell antigens [§]	–	–	+	–	–
TCR rearrangement	+	+	$\pm$	+	+
HTLV-1 antibody	–	+	–	–	$\pm$
Clinical course	Aggressive	Aggressive	Indolent	Aggressive	Chronic

*Pan-T antigens: CD2, CD3, CD5, CD7. Selective loss of one or more antigens may occur.

[†]NK-like T cells (but not true NK cells) express CD3.

[‡]Activation-associated antigens: CD38, CD71, HLA-DR.

[§]NK-cell antigens: CD16, CD56, CD57.

ATCL, adult T-cell leukemia/lymphoma; HTLV-1, human T-cell lymphotropic virus type 1; LGLD, large granular lymphoproliferative disorder; NK, natural killer; T-CLL, T-cell type of chronic lymphocytic leukemia; T-PLL, T-cell prolymphocytic leukemia; IL-2R, interleukin-2 receptor; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

For further information, the reader is referred to the corresponding Cases 22, 37, 39, and 41, respectively.

Immunophenotype

The immunophenotype of T-CLL is straightforward. It shows all pan-T-cell markers (CD2, CD3, CD5, and CD7), and the majority of cases belong to the helper/inducer subset (CD4+) (4,13–16). However, a few cases of the cytotoxic/suppressor subset have been reported (14,22). A Japanese study suggested that the loss of CD2 expression in T-CLL was associated with a poor prognosis (23). When T-cell receptor markers are analyzed, they usually express the T-cell receptor (TCR)_{ab} subtype and not the TCR_{gd} subtype (16). Thymic T-cell markers, such as terminal deoxynucleotidyl transferase and CD1a, are consistently negative in all cases.

The most important negative markers that help to distinguish T-CLL from other lymphoproliferative disorders are the B-cell markers (CD19, CD20, HLA-DR) and NK-cell markers (CD16, CD56, and CD57). In cases where CD8 is expressed, it is particularly important to include the NK-cell markers in the immunophenotypic panel to exclude NK or NK/T-cell lymphoproliferative disorders. True NK-cell leukemia shows absence of surface CD3 but presence of cytoplasmic CD3. A few cases of CD20-positive T-cell lymphoma have been reported (24), but CD20 has never been demonstrated in T-CLL cases.

Another important marker for differential diagnosis is CD25, which is seen in ATCL and occasionally in mycosis fungoides/Sézary syndrome (17,20). In Sézary syndrome and some peripheral T-cell lymphomas, CD7 is usually decreased as compared to other T-cell markers (such as CD3 and CD5).

Other than the above-mentioned markers (CD3, CD7, CD16, CD25, CD56, and CD57), the immunophenotype of T-CLL is similar to other T-cell lymphoproliferative disorders. It is hard to distinguish T-CLL from T-PLL by immunophenotyping, except that T-PLL usually has a variable subset expression (25). In other words, CD8-positive T-PLL is more frequently seen than CD8-positive T-CLL.

A few cases of S100-positive T-cell chronic lymphoproliferative diseases have been reported (26). Those cases have a very aggressive clinical course, with hepatosplenomegaly and central nervous system involvement. However, three of the four cases reported by Hanson et al. (26) expressed CD16, CD56, and CD57 and were probably cases of NK-cell leukemia/lymphoma. Another interesting immunophenotypic finding is that, even in B-CLL, oligoclonal expansion of the CD4+ CD57+ T-cell subset is consistently demonstrated (27).

Functionally, T-CLL cells frequently show decreased B-cell growth factor and B-cell differentiation factor, which can be the mechanism for hypogammaglobulinemia seen in some patients (28).

Comparison of Flow Cytometry and Immunohistochemistry

Antibodies of CD3, CD4, CD5, CD7, and CD8 are available for immunohistochemical staining and are sufficient for the identification of T-CLL. However, there are more antibodies available for flow cytometry, such as CD2, cytoplasmic

CD3, and CD25, that can be incorporated into a large panel for differential diagnoses of various T-cell lymphomas/leukemias.

Molecular Genetics

In the Mayo Clinic study (14), all T-CLL cases that had gene rearrangement analysis showed T-cell receptor b-chain gene rearrangement, and a few cases showed TCR g-chain gene rearrangement.

Recurrent cytogenetic abnormalities usually involve 14q11, 14q32, 7p15, and the long arm of chromosome 8 (14,29). The abnormalities are expressed in translocation, conversion, and isochromosome pattern. Because of the high frequency of abnormality in 14q32, the existence of a protooncogene at this locus was suspected (30). Finally, a putative oncogene, designated T-cell leukemia 1 (TCL)-1, has been identified at 14q32 (31). 14q11 is the location of the T-cell receptor ad locus, which can be fused with TCL-1 by translocation or inversion.

Gene transfer studies of TCL-1 suggest that its overexpression inhibits apoptosis, which is contributory to the neoplastic expansion of T cells (31). TCL-1 is normally expressed in CD4– CD8– immature thymocytes; therefore, its demonstration in T-CLL cells represents an inappropriate gene expression in leukemic cells that have a mature phenotype.

A Chinese study found that CC chemokine ligand 25 (CCL25) selectively enhanced resistance to TNF α -mediated apoptosis in T-CLL CD4+ cells (32). The CC chemokine receptor 9 (CCR9) on the T-CLL cells reacts with the CCL25/thymus-expressed chemokine (TECK) with resultant selective activation of Livin (a member protein of the inhibitor of apoptosis protein family) to enhance resistance to TNF α -mediated apoptosis (32).

The salient features for laboratory diagnosis of T-CLL are summarized in Table 6.20.2.

TABLE 6.20.2

Salient Features for Laboratory Diagnosis of T-CLL

1. An absolute lymphocyte count in the peripheral blood $>5 \times 10^9/L$
2. Leukemic cells: Predominantly small, mature-looking lymphocytes
3. Lymphocytosis in bone marrow $>30\%$
4. Expression of all pan-T-cell antigens: CD2, CD3, CD5, CD7
5. Mostly helper T-cell phenotype (CD4+ CD8–)
6. TCR gene rearrangement is present.
7. Common cytogenetic abnormalities: t(14;14)(q11;q32), inv(14;14)(q11;q32), involving TCL-1 oncogene and TCRA d.
8. TdT and CD1: Negative

T-CLL, T-cell chronic lymphocytic leukemia; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase.

Clinical Manifestations

The tumor cells in T-CLL and B-CLL are indistinguishable morphologically. Therefore, it is very important to identify the immunophenotype for their distinction, because these two entities are markedly different in the clinical course. B-CLL usually has an indolent course, whereas T-CLL is often highly aggressive with frequent skin and central nervous system involvement (1,4,14,33). Patients with T-CLL usually are refractory to the therapeutic regimen for B-CLL (14). In T-CLL, mild-to-moderate splenomegaly is present in about 40% and shotty adenopathy in 50% of cases (14).

In addition, the clinical course of T-CLL is similar to that of T-PLL, and so are their prognoses and cytogenetic abnormalities (25). Splenomegaly is more frequently seen in T-PLL, whereas lymphadenopathy is more common in T-CLL. Cases of T-CLL transforming to T-PLL have been reported; therefore, some cases may represent both entities (1).

REFERENCES

1. Witzig TE, Phyliky RL, Li CY, et al. T-cell chronic lymphocytic leukemia with a helper/inducer membrane phenotype: a distinct clinicopathologic subtype with a poor prognosis. *Am J Hematol*. 1986;21:139–155.
2. Tamura K, Sawada H, Izumi Y, et al. Chronic lymphocytic leukemia (CLL) is rare, but the proportion of T-CLL is high in Japan. *Eur J Haematol*. 2001;67:152–157.
3. Simpkins H, Kiproff DD, Davis JL III, et al. T cell chronic lymphocytic leukemia with lymphocytes of unusual immunologic phenotype and function. *Blood*. 1985;65:127–133.
4. Wong KF, Chan JKC, Sin VC. T-cell form of chronic lymphocytic leukaemia: a reaffirmation of its existence. *Br J Haematol*. 1996;93:157–159.
5. Chan WC, Foucar K, Morice WG, et al. T-cell large granular lymphocyte leukemia. In Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:272–273.
6. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukaemias. French-American-British (FAB) Cooperative Group. *J Clin Pathol*. 1989;42:567–584.
7. Matutes E, Catovsky D. CLL should be used only for the disease with B-cell phenotype. *Leukemia*. 1993;7:917–918.
8. Matutes E, Catovsky D. Similarities between T-cell chronic lymphocytic leukemia and the small-cell variant of T-prolymphocytic leukemia. *Blood*. 1996;87:3520–3521.
9. Foon KA, Gale RP. Is there a T-cell form of chronic lymphocytic leukemia? *Leukemia*. 1992;6:867–868.
10. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1369.
11. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November 1997. *Mod Pathol*. 2002;13:193–207.
12. Swerdlow SH, Campo E, Harris NL, et al. eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008.
13. Neame PB, Soamboonsrup P, Giesbrecht J. T-cell form of chronic lymphocytic leukemia. *Leukemia*. 1993;7:916–917.
14. Hoyer JD, Ross CW, Li CY, et al. True T-cell chronic lymphocytic leukemia: a morphologic and immunophenotypic study of 25 cases. *Blood*. 1995;86:1163–1169.
15. Hanson CA, Hoyer JD, Li CY, et al. Similarity between T-cell chronic lymphocytic leukemia and the small-cell variant of T-prolymphocytic leukemia. *Blood*. 1996;87:3520–3521.
16. Soma L, Cornfield DB, Prager D, et al. Unusually indolent T-cell prolymphocytic leukemia associated with a complex karyotype: is this T-cell chronic lymphocytic leukemia? *Am J Hematol*. 2002;71:224–226.
17. Ralfkiaer E, Cerroni L, Sander CA, et al. Mycosis fungoides. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:296–298.
18. Ralfkiaer E, Willemze R, Whittaker SJ. Sézary syndrome. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:299.
19. Pawson R, Matutes E, Brito-Babapulle V, et al. Sézary cell leukemia: a distinct T-cell disorder or a variant form of T-prolymphocytic leukemia. *Leukemia*. 1997;11:1009–1013.
20. Ohshima K, Jaffe ES, Kikuchi. Adult T-cell leukaemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:281–284.
21. Porwit A, Djokic M. T-cell prolymphocytic leukemia. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:513–520.
22. Phyliky RL, Li CY, Yam LT. T-cell chronic lymphocytic leukemia with morphologic and immunologic characteristics of cytotoxic/suppressor phenotype. *Mayo Clin Proc*. 1983;58:709–720.
23. Tamura K, Sagawa K, Satoh H, et al. Poorly expressed CD2 antigen on the leukemic cells of adult T-cell leukemia and chronic lymphocytic leukemia of T-cell lineage. *Leuk Res*. 1989;13:93–99.
24. Sun T, Akalin A, Rodacker M, et al. CD20 positive T cell lymphoma: is it a real entity? *J Clin Pathol*. 2004;57:442–444.
25. Catovsky D, Müller-Hermelink HK, Ralfkiaer E. T-cell prolymphocytic leukemia. In Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:270–271.
26. Hanson CA, Bockenstedt PL, Schnitzer B, et al. S100-positive, T-cell chronic lymphoproliferative disease: an aggressive disorder of an uncommon T-cell subset. *Blood*. 1991;78:1803–1813.
27. Serrano D, Monteiro J, Allen SL, et al. Clonal expansion within the CD4+ CD57+ and CD8+ CD57+ T-cell subsets in chronic lymphocytic leukemia. *J Immunol*. 1997;158:1482–1489.
28. Raziuddin S, Sheikh A, Latif AA. T-cell chronic lymphocytic leukemia: T-cell function and lymphokine secretion. *Cancer*. 1992;69:1146–1152.
29. Bartlett NL, Long DL. T-small lymphocyte disorders. *Semin Hematol*. 1999;36:164–170.
30. Reed JC. Molecular biology of chronic lymphocytic leukemia. *Semin Oncol*. 1998;25:11–18.
31. Fu TB, Virgilio L, Narducci MG, et al. Characterization and localization of the TCL-1 oncogene product. *Cancer Res*. 1994;54:6297–6301.
32. Zhang Q, Xiong J, Jin Y, et al. CC chemokine ligand 25 enhances resistance to apoptosis in CD4+ T cells from patients with T-cell lineage acute and chronic lymphocytic leukemia by means of Lck activation. *Cancer Res*. 2004;64:7579–7587.
33. Nousari HC, Kimyai-Asadi A, Huang CH, et al. T-cell chronic lymphocytic leukemia mimicking dermatomyositis. *Int J Dermatol*. 2000;39:144–146.

CASE 21

Small Lymphocytic Lymphoma

CASE HISTORY

A 75-year-old man presented with shortness of breath and productive cough for several months. The patient had a long history of chronic obstructive pulmonary disease. Chest x-ray examination revealed right upper lobe and perihilar infiltrate. He was then treated with antibiotics and oxygen. Physical examination showed multiple lymphadenopathy involving occipital, posterior triangle of the neck, anterior triangle, submandibular, submental, supraclavicular, bilateral axillary, and right inguinal regions. Computed axial tomography scan also detected large mediastinal masses and complete opacification of the adjacent right lung field. Abdomen revealed possible hydronephrosis of the left kidney. Organomegaly was not found in the patient.

Arterial blood gas analysis showed pH 7.54, pCO₂ 29.8, pO₂ 57, and oxygen saturation 93%. Blood chemistry was unremarkable except for a high level of lactate dehydrogenase (LDH) (292 U/dL). A hematology workup revealed a total leukocyte count of 3,000/mL with 27% neutrophils, 71.4% lymphocytes, and 1.1% monocytes. No leukemic cells were detected in the peripheral blood. His hematocrit was 43% and hemoglobin 14 g/dL. A sputum culture grew pseudomonas.

An axillary lymph-node biopsy showed features of small-cell lymphoma. The patient continued to receive oxygen and antibiotic treatment. In addition, chemotherapy with rituximab, fludarabine, and cyclophosphamide was started and was complicated with hypotension. This was considered to be the side effect of rituximab, which was then withheld. The patient tolerated one cycle of chemotherapy without further complication. His oxygenation slowly improved, and chest x-ray also showed improvement. The patient was discharged to the nursing home care unit with continued monitoring by the Hematology/Oncology Service.

FLOW CYTOMETRIC FINDINGS

CD5 98%, CD19 85%, CD19/CD5 84%, CD20 77%, CD10 1%, CD23 91%, FMC-7 13%, CD19/k 88%, CD19/l 4% (Fig. 6.21.1).

IMMUNOHISTOCHEMICAL FINDINGS

Immunohistochemical stains showed that the tumor cells were positive for CD20, but negative for CD3 and cyclin D1.

DISCUSSION

Small lymphocytic lymphoma (SLL) is a small B-cell lymphoma and the tissue phase of chronic lymphocytic leukemia (CLL) (1,2). The term SLL, as defined by the International Workshop on Chronic Lymphocytic Leukemia (IWCLL), is reserved for those nonleukemic cases with lymphadenopathy, no cytopenia due to bone marrow tumor infiltration, and <5,000/mL B cells in the peripheral blood (3). However, even in those cases, the bone marrow and peripheral blood may be eventually involved. In patients showing both tissue and leukemic phases, the appropriate term should be CLL/SLL. SLL and CLL have identical immunophenotypes and are considered the same disease in the World Health Organization (WHO) classification (1,2). This classification is distinguished from the Revised European-American Classification of Lymphoid Neoplasms (REAL classification) (4) by separating prolymphocytic leukemia from CLL/SLL. CLL/SLL accounts for 90% of chronic lymphoid leukemia in the United States and Europe (1). It comprises 6.7% of non-Hodgkin lymphoma (1,2). The incidence is 5.17 per 100,000 person-years (5).

Morphology

In the lymph node, the normal architecture is usually totally effaced by small neoplastic lymphoid cells. In a small percentage of cases, an interfollicular pattern may be present (1,2). The tumor cells are small lymphoid cells with regular nuclei and a clumped chromatin pattern. Nucleoli are usually not present or inconspicuous. The cytoplasm is scant.

The most characteristic pattern is the presence of proliferation centers or pseudofollicles, which are present in 90% of SLL cases (Fig. 6.21.2) (6). This structure is best detected under low-power magnification as a poorly defined pale stained area. It is composed of prolymphocytes and paraimmunoblasts surrounded by a small lymphocyte background (Fig. 6.21.3). Prolymphocytes are slightly larger than the small lymphocytes, with a dispersed chromatin pattern and inconspicuous nucleoli. Paraimmunoblasts are larger than prolymphocytes, with dispersed chromatin pattern and one or more conspicuous nucleoli. A pseudofollicle should be distinguished from a residual germinal center, which can be seen in mantle cell lymphoma (MCL) and nodal marginal zone B-cell lymphoma. A germinal center is well defined and is composed of centrocytes, centroblasts, and macrophages, forming a pattern of polarity. In some instances, a cluster of parafollicular/monocytoid B cells may also mimic the proliferation center (6). The number and size of pseudofollicles

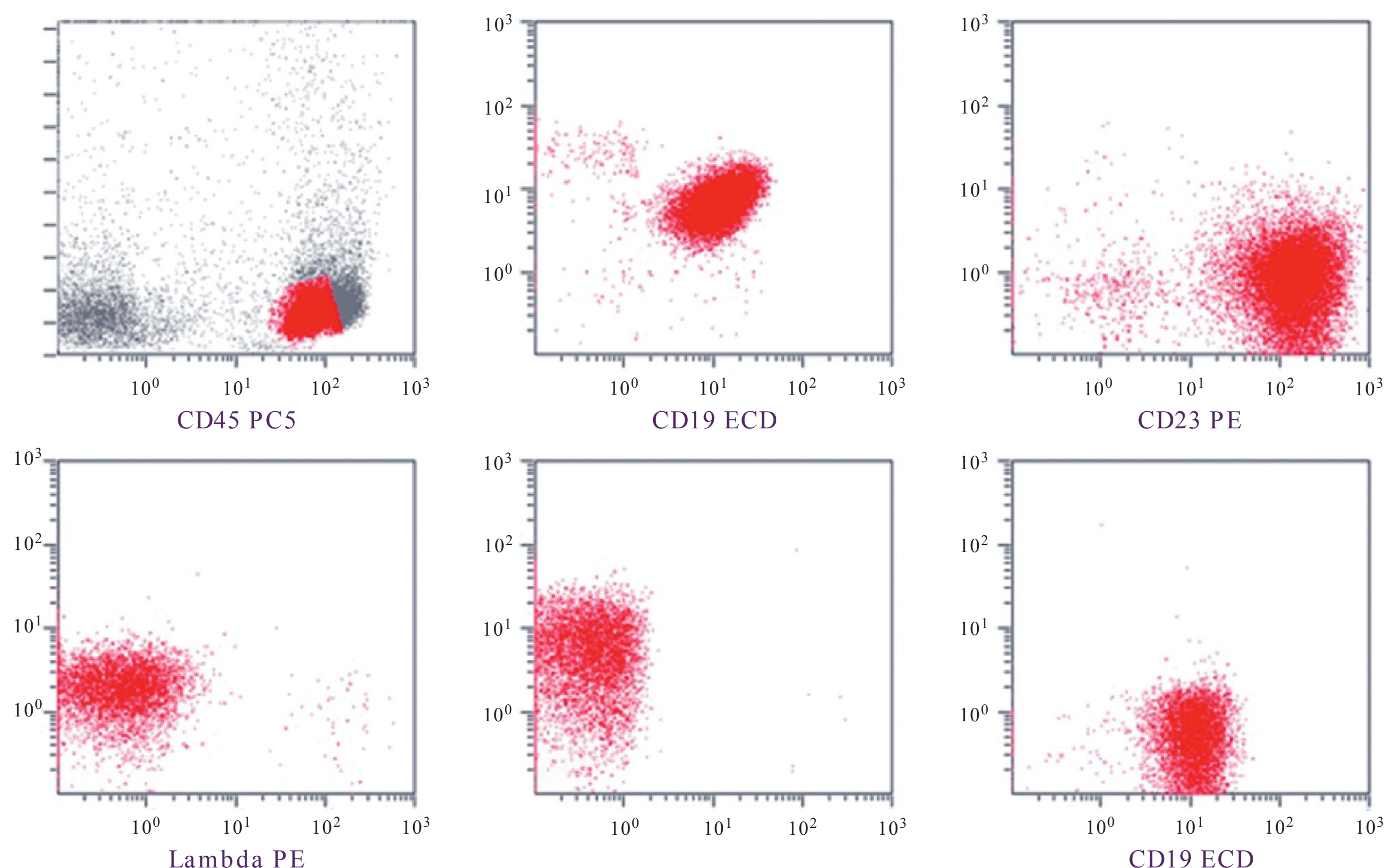


FIGURE 6.21.1 Flow cytometric analysis shows dual CD19/CD5 staining, strong positive reactions for CD23 and CD20, but dim k staining. 1, FMC-7 and CD10 are negative. SS, side scatter; PC5, phycoerythrin-cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ECD, phycoerythrin-Texas Red.

differ from case to case, but several studies found that this variation has no correlation with clinical condition and immunophenotype (7,8). However, one recent study found that the presence of expanded proliferation center (broader than a 20× field) and a high proliferation rate

(either 2.4 mitosis per proliferation center or Ki-67 >40% per proliferation center) predict poor outcome. These cases, designated accelerated SLL/CLL, are associated with higher serum LDH levels and higher percentage of ZAP-70 than nonaccelerated cases (9).

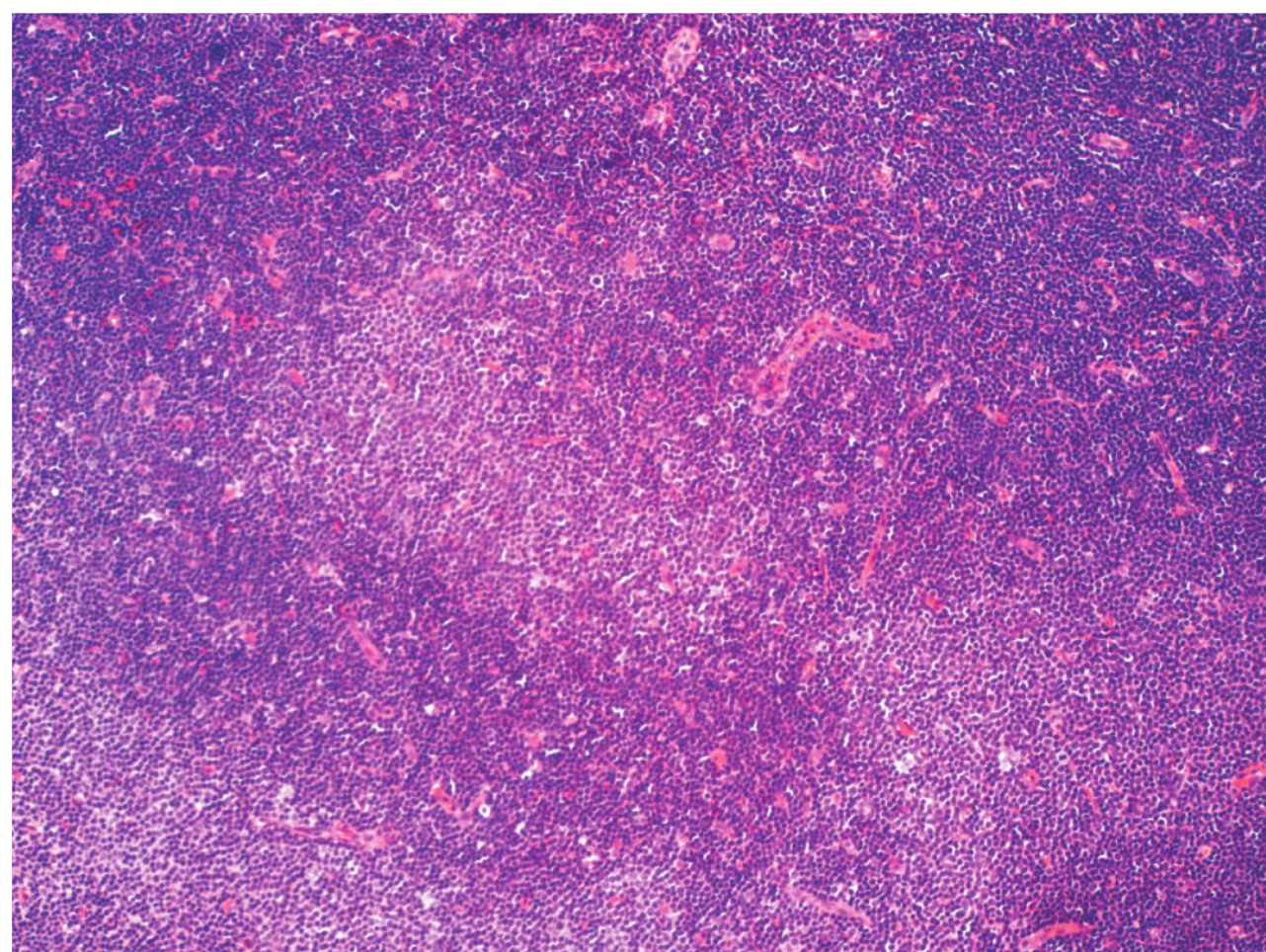


FIGURE 6.21.2 Lymph node biopsy shows total effacement of normal architecture by small lymphocyte infiltration. There are pale-stained areas, representing the proliferation centers. Hematoxylin and eosin, 10× magnification.

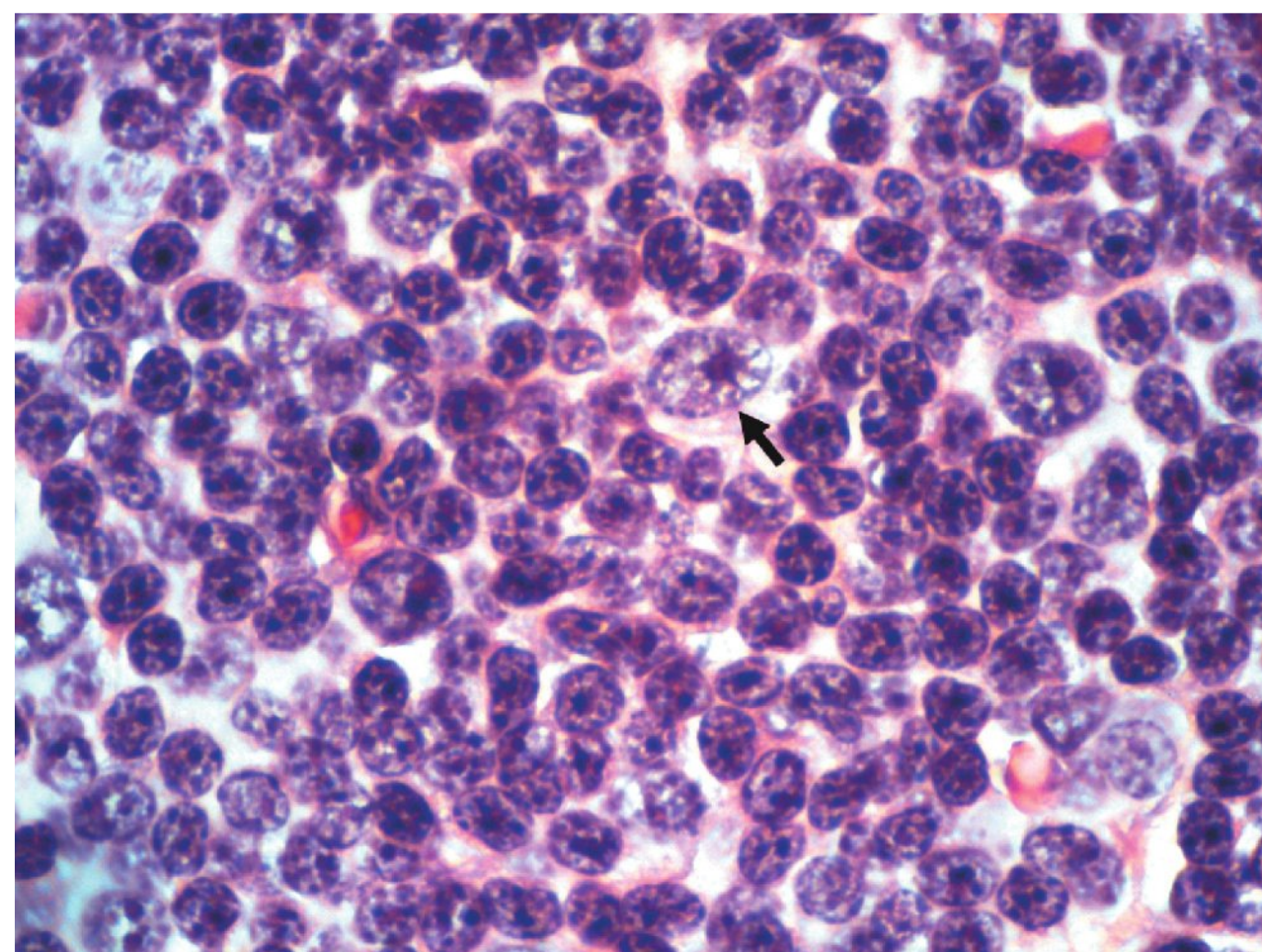


FIGURE 6.21.3 Lymph node biopsy in a case with prolymphocytoid transformation reveals predominance of prolymphocytes with scattered paraimmunoblasts (arrow). Hematoxylin and eosin, 100× magnification.

SLL should be distinguished from other small-cell lymphomas, particularly the diffuse type of MCL, which has an immunophenotype similar to that of SLL. MCL, however, does not have pseudofollicles, and the tumor cells are usually more irregular than those of SLL (6).

In the spleen, the white pulp is primarily involved, but red pulp infiltration is also present (7). Pseudofollicles may be seen in the spleen, but they are not as conspicuous as they are in the lymph nodes. The bone marrow may show nodular, interstitial (Fig. 6.21.4), or diffuse infiltration pattern (or a combination of the three), but paratrabecular infiltration is seldom encountered (7). A diffuse infiltration pattern is usually associated with a poor prognosis (1,2). Extranodal involvement is mainly seen in the orbit and lungs. The diagnostic morphologic features of SLL are listed in Table 6.21.1.

When the cells of the pseudofollicles predominate and proliferate diffusely with resultant nodal replacement, the subtype is designated as a paraimmunoblastic variant of SLL/CLL by Pugh et al. (10) and the tumor-forming subtype of B-CLL in the Kiel classification (11) (Fig. 6.21.5). Paraimmunoblasts should be distinguished from immunoblasts, because the proliferation of immunoblasts represents large B-cell transformation. The immunoblasts have more vesicular nuclear chromatin, larger and more prominent nucleoli, and darker basophilic (Giemsa), amphophilic (hematoxylin and eosin), or pyronophilic methyl green pyronin cytoplasm than the paraimmunoblasts have (11).

The recognition of this subtype, although rare, is important because it is frequently mistaken for other types of large-cell lymphomas (7), particularly the blastic and large-cell variants of MCL (12). This subtype should also be distinguished from prolymphocytic transformation of CLL, which usually shows a large number of prolymphocytes in the peripheral blood and a history of CLL. Histologically, paraimmunoblasts are not a component of prolymphocytic transformation (7). Prolymphocytoid transformation is encountered in approximately 15% of CLL cases (13).

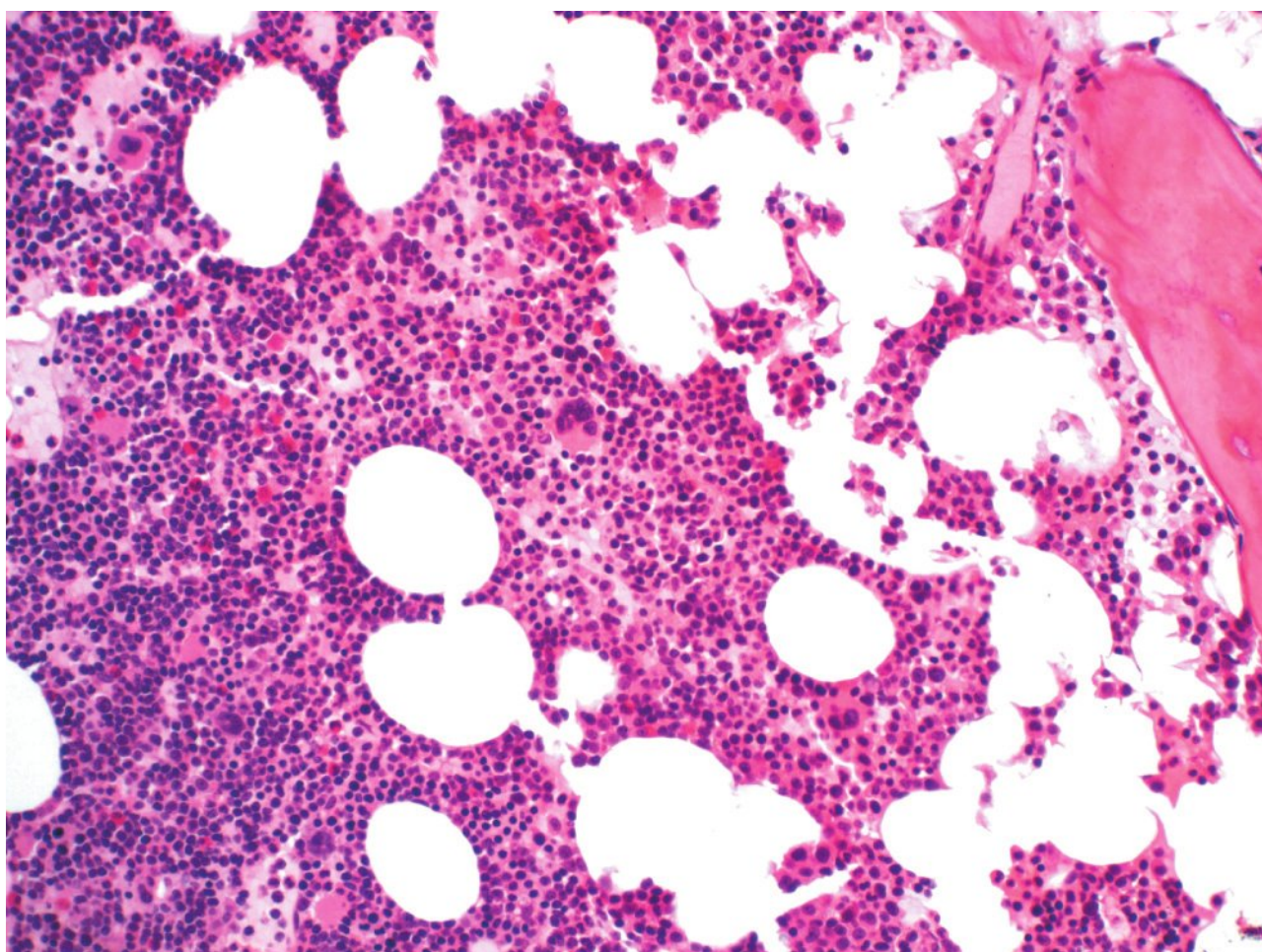


FIGURE 6.21.4 Bone marrow biopsy shows interstitial small lymphocytic infiltration. Hematoxylin and eosin, 20× magnification.

TABLE 6.21.1	
Characteristic Morphologic Features in SLL	
Histologic pattern	Diffuse small lymphocytic infiltration replacing normal architecture
Cytology	Small, uniform, regular shaped, mature-looking lymphocytes with dense, clumped chromatin and no conspicuous nucleolus
Specific feature	Presence of proliferation centers

Transformation to large B-cell lymphoma or the so-called Richter syndrome is seen in approximately 3% of patients with CLL (14). These large-cell lymphomas are usually diffuse large B-cell lymphoma, including the centroblastic and immunoblastic variants (1,2,15). Histologically, the transformed large-cell lymphoma shows confluent sheets of large tumor cells (Fig. 6.21.6), in contrast to prolymphocytoid transformation, in which the prolymphocytes intermix with the small lymphoid tumor cells. A proliferation fraction exceeding 30%, as demonstrated by Ki-67 staining, is indicative of transformation to large B-cell lymphoma (Fig. 6.21.7) (13).

Immunophenotype

As B-cell neoplasms, SLL cells express most of the B-cell markers (CD19, CD20, CD21, CD23, CD24, CD79a, HLA-DR, and monoclonal surface immunoglobulin [Ig] M and/or IgD), except for CD22, which is usually weak or absent on SLL cells (1,2,6,17,18). However, the most characteristic feature in SLL is the coexpression of CD19 (CD20)/CD5 (6). Nevertheless, this dual staining is present also in CLL and MCL. CLL is the leukemic counterpart of SLL; thus,

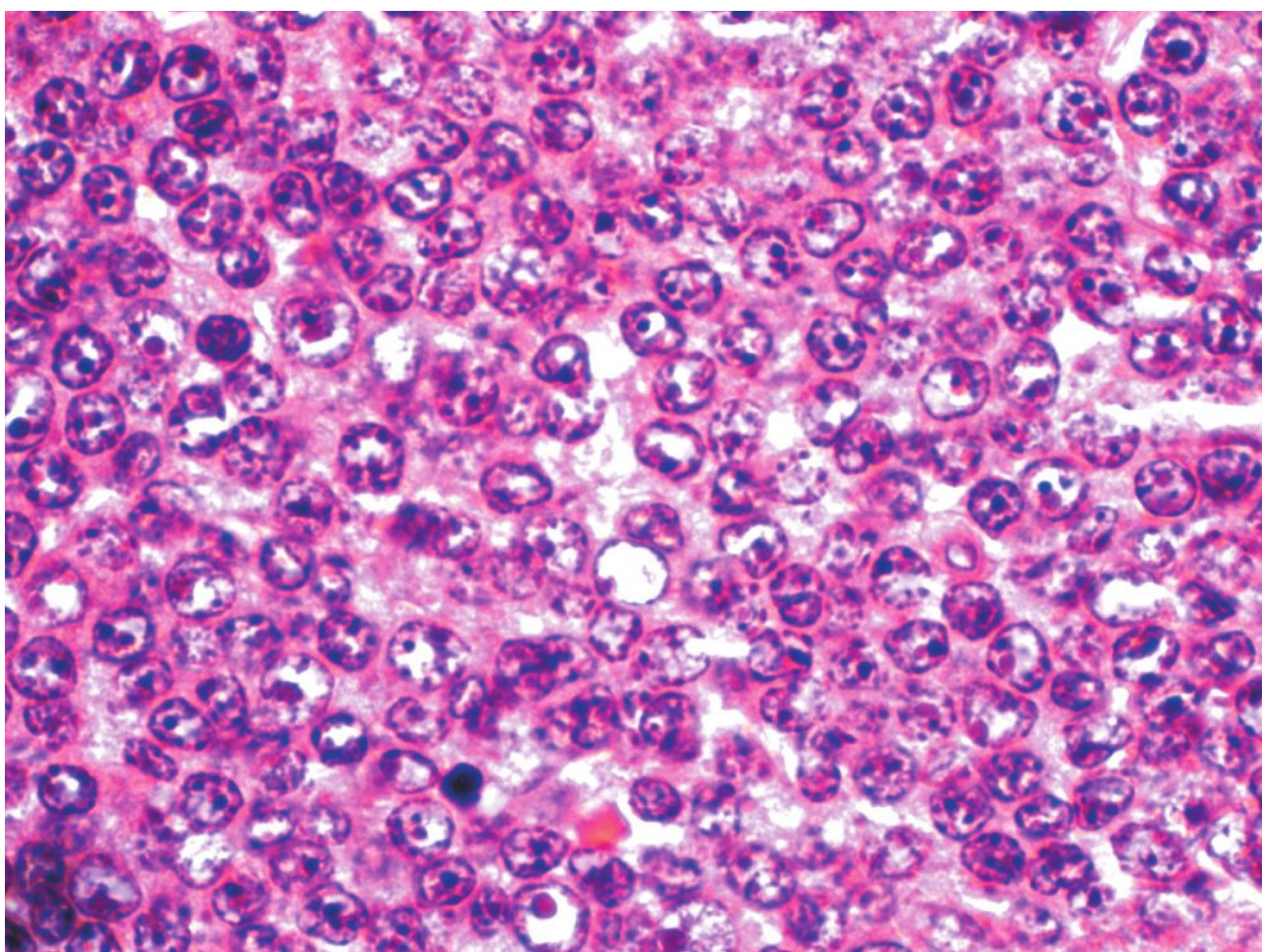


FIGURE 6.21.5 Lymph node biopsy in a case of paraimmunoblastic variant reveals exclusively paraimmunoblastic infiltration. Hematoxylin and eosin, 100× magnification.

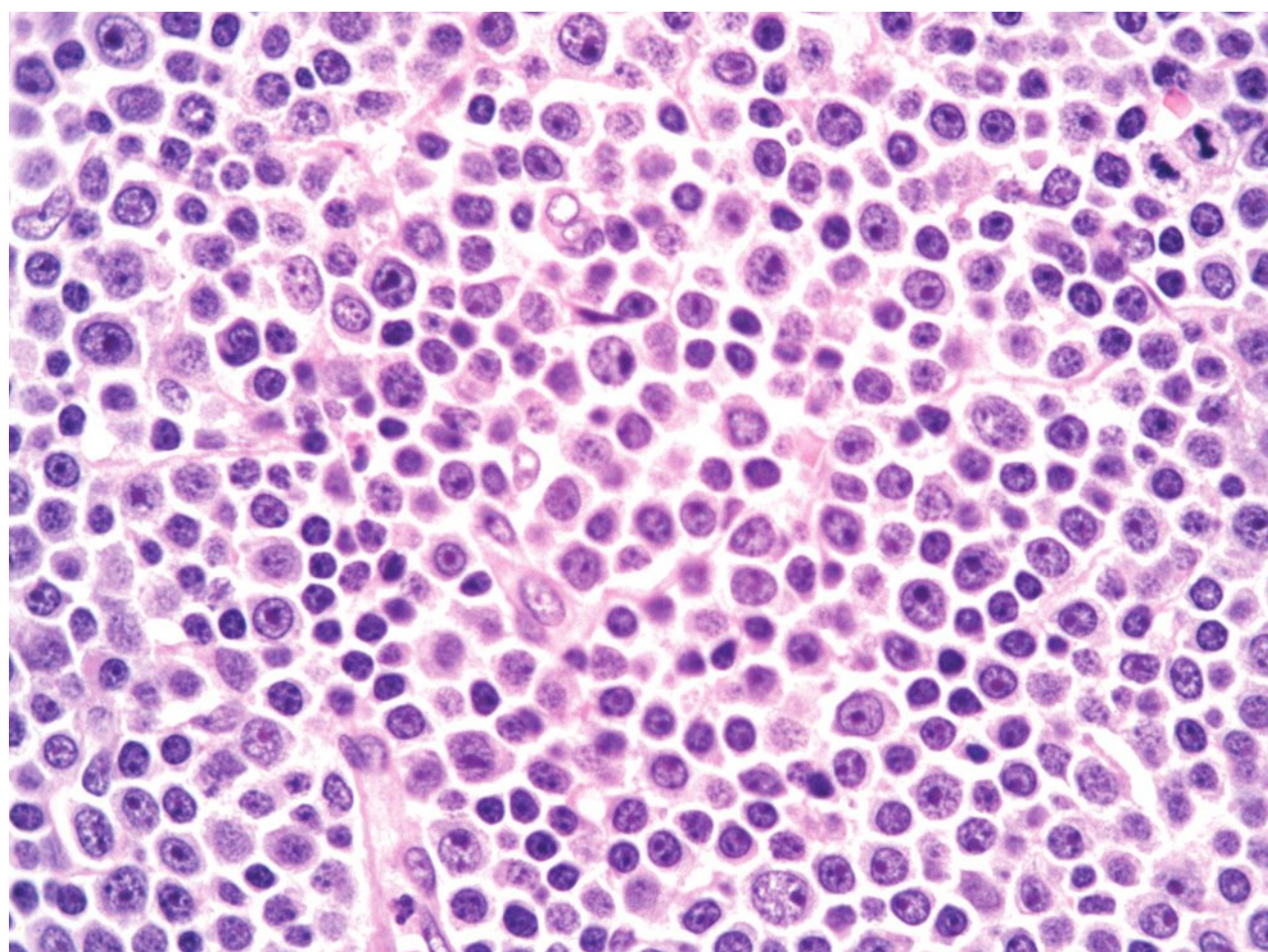


FIGURE 6.21.6 Lymph node biopsy in a case of Richter transformation shows scattered atypical large lymphoid cells on a small lymphocyte background. Hematoxylin and eosin, 60× magnification.

no routine surface marker can help distinguish them (19). Although the adhesion markers CD11a/CD18 are present on the cells of SLL (but not on those of CLL) (20), adhesion molecules are not routinely tested in clinical laboratories. A recent study showed that the chemokine receptors CXCR4 and CCR7 are expressed at higher levels in peripheral CLL cells than SLL cells in the lymph nodes (21).

SLL/CLL usually shows dim fluorescence intensity of surface Ig, and the intensity of CD19 is stronger than that of CD20 in SLL/CLL. These features may help distinguish SLL from MCL, which shows moderate intensity of surface Ig and stronger intensity of CD20 than of CD19 (17). However, because of the availability of CD23, the distinction between these two tumors no longer depends on the staining intensity of different antibodies. The SLL/CLL cases are usually positive for CD23, whereas the MCL cases are just

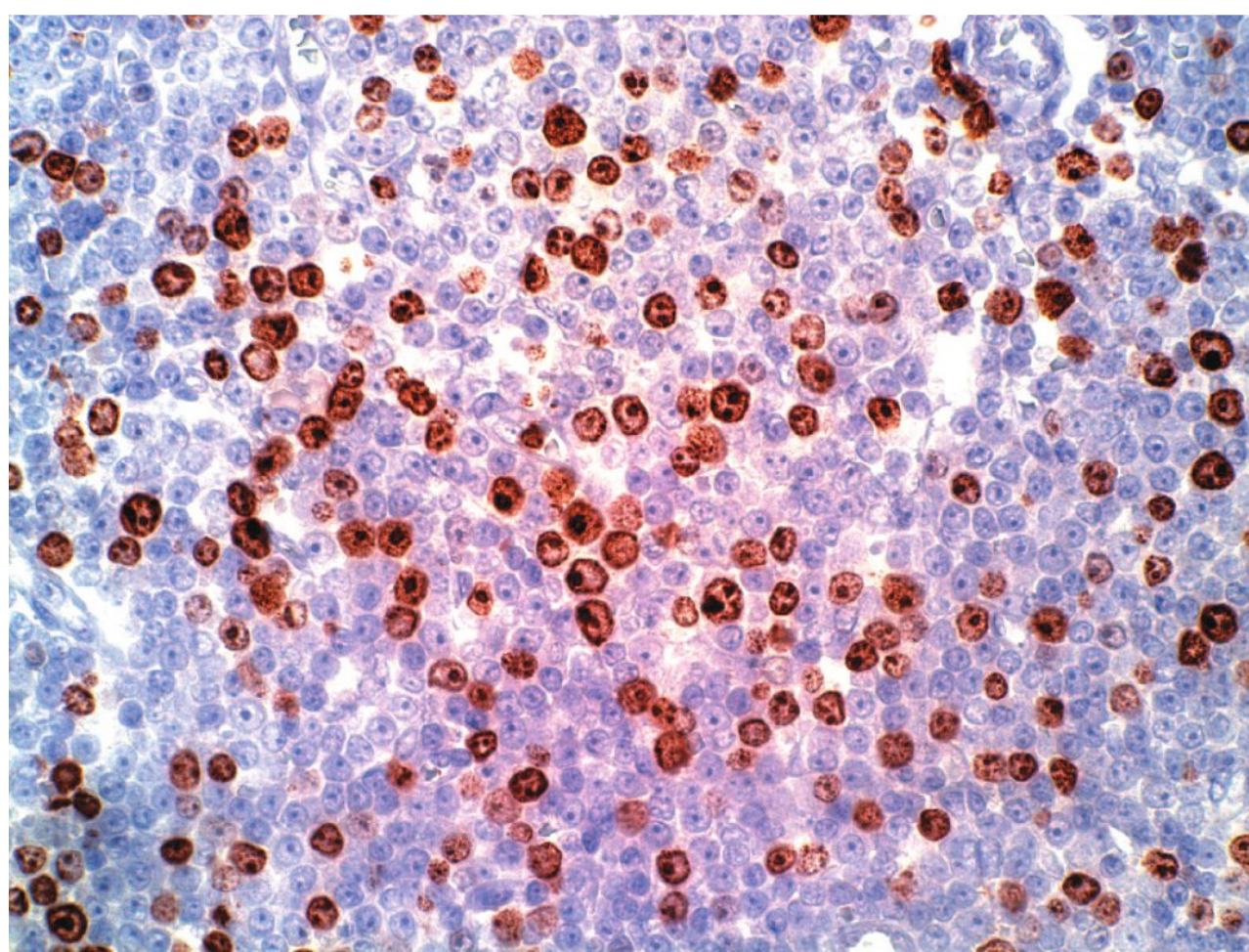


FIGURE 6.21.7 Lymph node biopsy in a case of Richter transformation reveals large numbers of Ki-67 stained cells. Immunoperoxidase, 40× magnification.

the opposite (22–25). Nevertheless, about 10% to 15% of MCL is positive for CD23 (7). A higher percentage of CD23-positive MCL cases have been reported in some recent series (26,27). In those cases, a positive cyclin D1 (*bcl-1* gene product) staining may help to establish the diagnosis of MCL (5,24,26). SLL generally shows no *bcl-1* translocation except for the paraimmunoblastic variant of SLL (12).

CD23 is persistent on large-cell lymphoma cells transformed from SLL/CLL, but it remains negative in the blastic variant of MCL; therefore, it helps to distinguish these two entities even when they are transformed (28).

Because CD5 monoclonal antibody is now available for paraffin sections, diagnosis of SLL by immunohistochemistry has become feasible (29). In the plasmacytoid subtype of SLL, which is now designated by WHO as Waldenström macroglobulinemia/lymphoplasmacytic lymphoma, CD5 is consistently negative (11). In a small percentage of SLL/CLL cases, CD5 is absent (30). Some of those CD5-negative cases could be the result of a negative conversion after chemotherapy (personal observation). In Japan, the incidence of CLL/SLL is low, but CD5-positive B-cell lymphomas/leukemias usually show a poor overall prognosis (31). CD5 has also been demonstrated in a small group of *de novo* diffuse large B-cell lymphomas and in a few cases of marginal zone B-cell lymphoma and Burkitt lymphoma (32–34).

Other markers helpful in differential diagnosis include FMC-7 and CD43. FMC-7, used in flow cytometry, is positive in MCL but negative or weakly positive in SLL (22,25). CD43, used in immunohistochemical staining, is positive in both MCL and SLL, but it can help to distinguish several CD43-negative lymphomas, such as follicular lymphoma, marginal zone lymphoma, and lymphoplasmacytic lymphoma (24,25). CD11c, a monocytic marker that is positive for hairy cell leukemia, was also positive in 83% of SLL cases in one study (25). SLL may also show selective loss of pan-B-cell antigen or negative surface Ig (35).

A novel combined silver nitrate and immunoperoxidase technique has been reported to be useful in distinguishing SLL/CLL from reactive lymphoid hyperplasia in the spleen (36). This technique helps to demonstrate trabecular infiltration, subendothelial infiltration, and prominent sinus involvement in SLL/CLL but not in lymphoid hyperplasia.

Comparison between Flow Cytometry and Immunohistochemistry

The major immunophenotype of SLL is CD19+/CD20+, CD5+, CD23+, and FMC-7–, which distinguishes SLL from MCL and other non-Hodgkin lymphomas. This immunophenotype can be easily identified by flow cytometry. Nevertheless, CD23 can be present in MCL cases, and FMC-7 can be positive in SLL cases by flow cytometry (37). Immunohistochemistry can also stain for CD20, CD5, CD23, but not FMC-7. However, *bcl-1* can be demonstrated by immunohistochemical stain and is a more powerful tool to distinguish MCL from SLL.

The presenting symptoms in the current case were those of pneumonia, but multiple lymphadenopathy was discovered during physical examination. The diagnosis of SLL was established on the basis of the morphology of the

TABLE 6.21.2

Salient Features of Flow Cytometric Diagnosis of SLL

1. Monoclonal surface immunoglobulin with dim fluorescence.
2. Dual staining of CD19 (CD20) and CD5 is characteristic.
3. Positive CD23 distinguishes SLL from MCL.
4. Positive B-cell antigens: CD19, CD20, CD21, CD24, CD79a, and HLA-DR.
5. Important negative antigens: CD22, CD10, FMC-7 (most cases).
6. Immunoglobulin-negative, B-cell antigen-positive pattern may be present.
7. Selective loss of pan-B-cell antigen may be present.

lymph node, showing a small-cell lymphoma with multiple pseudofollicles. The flow cytometric analysis and immunohistochemical staining of the lymph node further confirmed this diagnosis. Because the lymphoma involved the mediastinum and spread to the adjacent lung field, lymphoma obstruction of the bronchus was considered the predisposing factor of his pneumonia. The improvement of pulmonary symptoms after chemotherapy supports this assumption. Small-cell lymphoma usually has an indolent clinical course; therefore, when the patient becomes symptomatic, the disease is already in the late stage. In this case, the extensive multiple lymph node involvement suggests that the patient might have had the disease for years rather than months. It was the superimposed infection of the lung that drew the attention of the clinicians to make the diagnosis of lymphoma.

The diagnostic features of SLL by flow cytometry are summarized in Table 6.21.2.

Molecular Genetics

In terms of genotyping, Ig heavy- and light-chain gene rearrangements are usually demonstrated in SLL cases. When SLL cases transform into large-cell lymphoma (Richter syndrome), these two tumors often share the same genotype (14,38,39). However, some cases may show nonidentical rearranged bands. This phenomenon may be explained by the existence of two different clones or by the presence of heavy-chain gene switching, postrearrangement gene deletion, and somatic mutation after neoplastic transformation (40–42).

Approximately 40% to 50% of SLL cases show no somatic mutation of the Ig V_H genes, whereas 50% to 60% of cases show somatic mutation (1,2). Those cases with unmutated V_H genes are associated with positive CD38 (>30%) and a poor prognosis (43).

SLL usually shows no cytogenetic abnormalities by karyotyping but about 80% of CLL/SLL cases have cytogenetic aberrations detected by fluorescent in situ hybridization technique (1,2). The relatively frequent aberrations

include deletions at 13q14 (50%), trisomy 12 (20%), deletions at 11q22-23 (20%), deletions at 17p13 (10%), and deletions at 6q21 (5%) (1,18,44). One study of 55 cases of SLL showed that del(6)(q21;q23) was the most common recurring cytogenetic abnormality associated with the presence of large prolymphocytoid cells in the peripheral blood, but the clinical course of this subtype does not differ from the typical cases of SLL (45). Deletion of 17q13 is associated with p53 deletion. Trisomy 12 is associated with unmutated Ig V_H genes.

Translocation of bcl-2 is not seen in SLL (44), but bcl-2 protein was demonstrated in the bone marrow from 8 of 10 cases of SLL/CLL in one study (46). Translocation of bcl-1 is also not encountered in SLL except for its paraimmunoblastic variant (12). However, recent studies raised the possibility that those so-called paraimmunoblastic variants may well be the large-cell variants of MCL (34).

Leukemic phase is more commonly seen in SLL than in other lymphomas. Flow cytometry is usually able to detect the lymphoma cells in the blood. When the number of neoplastic cells is small, a data analysis system (Kolmogorov–Smirnov test) was advocated for clonal excess determination (47). However, the availability of the polymerase chain reaction has made the above technique obsolete (48).

Recently gene expression profiling provides a powerful tool for differential diagnosis as well as for stratifying prognostic subgroups of the same lymphoma. Genes associated with cell adhesion, angiogenesis, and inhibition of apoptosis were found to be upgraded in SLL (46). SLL was also found to share with CLL a common signature, including overexpression of L-selectin, P-selectin, titin, interleukin-4 receptor, CCR, adenylate kinase, diacylglycerol kinase, cyclin D2, and bcl-2 (49). However, the transcriptional profile of SLL was clearly distinguished from that of MCL and splenic marginal zone lymphoma (49). Another study of 120 genes showed distinctive gene expression profiling patterns among SLL, reactive lymph nodes, follicular lymphoma, and MCL (50).

Clinical Manifestations

Most SLL patients are >50 years old, and the male-to-female ratio is about 2:1 (1). Patients are often asymptomatic, and the initial diagnosis is usually due to the presence of painless lymphadenopathy. Some patients may have symptoms of fatigue, autoimmune hemolytic anemia, infections, splenomegaly, hepatomegaly, or extranodal infiltration (1,2,7). Anemia, weight loss, and night sweats are present in 15% to 43% of SLL cases (7).

The patient may present with only lymphadenopathy at the beginning, but bone marrow and peripheral blood involvement develop eventually. A small percentage of patients may have a low level of monoclonal gammopathy.

When Richter transformation is present, the patient's clinical condition usually changes abruptly, with marked increase of peripheral lymphocyte counts, fever, and the involvement of the central nervous system or other extranodal sites (15). When it occurs, the patient runs a rapidly downhill clinical course and dies within 6 months.

Nodal involvement is seen in 85% of patients at presentation (7). Only 6% of patients are in stage 1 or 2 at the time

of diagnosis, whereas 73% of patients have bone marrow involvement (>30% lymphocytes) and 30% have extranodal soft tissue involvement.

The 5-year overall survival rate varies from 51% to 66%, and failure-free survival varies from 23% to 25% in two different series (7,51). The adverse predictors for overall survival include age >60 years, B symptoms, elevated serum LDH, low hemoglobin (<11 g/dL), and high International Prognostic Index Score (>3) (51). SLL/CLL patients have a high frequency for developing a second malignancy, which is the major cause of death in this population (52). One study on the veteran population showed that most second malignancies are nonlymphoid tumors (52).

REFERENCES

- Müller-Hermelink HK, Catovsky D, Montserrat E, et al. Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:127–130.
- Müller-Hermelink HK, Montserrat E, Catovsky D, et al. Chronic lymphocytic leukaemia/small lymphocytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:180–182.
- Hellek M, cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia (IWCLL) updating the National cancer Institute-Working Group (NCI-WG) 1996 guidelines. *Blood*. 2008;111:5446–5456.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
- Morton LM, Wang SS, Devesa SS, et al. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. *Blood*. 2006;107:265–276.
- Swerdlow SH. Small B-cell lymphomas of the lymph nodes and spleen. Practical insights to diagnosis and pathogenesis. *Mod Pathol*. 1998;12:125–140.
- Ben-Ezra J. Small lymphocytic lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:773–787.
- Asplund SL, McKenna RW, Howard MS, et al. Immunophenotype does not correlate with lymph node histology in chronic lymphocytic leukemia/small lymphocytic lymphoma. *Am J Surg Pathol*. 2002;26:624–629.
- Giné E, Martinez A, Villamor N, et al. Expanded and highly active proliferation centers identify a histological subtype of chronic lymphocytic leukemia (“accelerated” chronic lymphocytic leukemia) with aggressive clinical behavior. *Hematologica*. 2010;95(9):1526–1533.
- Pugh WC, Manning JT, Butler JJ. Paraimmunoblastic variant of small lymphocytic lymphoma/leukemia. *Am J Surg Pathol*. 1988;12:907–917.
- Feller AC, Diebold J. *Histopathology of Nodal and Extranodal Non-Hodgkin’s Lymphoma*. 3rd ed. Berlin: Springer; 2004: 23–29.
- Grosso LE, Kelley PD. Bcl-1 translocations are frequent in the paraimmunoblastic variant of small lymphocytic lymphoma. *Mod Pathol*. 1998;11:6–10.
- Melo JV, Catovsky D, Gregory WM, et al. The relationship between chronic lymphocytic leukaemia and prolymphocytic leukaemia. IV. Patterns of evolution of “prolymphocytoid” transformation. *Br J Haematol*. 1986;64:77–86.
- Robertson LE, Pugh W, O’Brien S, et al. Richter’s syndrome: a report on 39 patients. *J Clin Oncol*. 1993;11:1985–1989.
- Gilles FJ, O’Brien SM, Keating MJ. Chronic lymphocytic leukemia in (Richter’s) transformation. *Semin Oncol*. 1998;25:117–135.
- Shin HJC, Caraway NP, Katz RL. Cytomorphologic spectrum of small lymphocytic lymphoma in patients with an accelerated clinical course. *Cancer Cytopathol*. 2003;99:293–300.
- Thakhi A, Etinger M, Myles J, et al. Flow cytometric immunophenotyping of non-Hodgkin’s lymphomas and related disorders. *Cytometry*. 1996;25:113–124.
- Jennings CD, Foon KA. Recent advances in flow cytometry. Application to the diagnosis of hematologic malignancy. *Blood*. 1997;90:2863–2892.
- Batata A, Chen B. Relationship between chronic lymphocytic leukemia and small lymphocytic lymphoma. A comparative study of membrane phenotypes in 270 cases. *Cancer*. 1992;70:625–632.
- Ben-Ezra J, Burke JS, Swartz WG, et al. Small lymphocytic lymphoma. A clinicopathologic analysis of 268 cases. *Blood*. 1989;73:579–587.
- Ghobrial IM, Bone ND, Stenson MJ, et al. Expression of the chemokine receptors CXCR4 and CCR7 and disease progression in B-cell chronic lymphocytic leukemia/small lymphocytic lymphoma. *Mayo Clin Proc*. 2004;79:318–325.
- Kilo MN, Dorfman DM. The utility of flow cytometric immunophenotypic analysis in the distinction of small lymphocytic lymphoma/chronic lymphocytic leukemia from mantle cell lymphoma. *Am J Clin Pathol*. 1996;105:451–457.
- Kumar S, Green GA, Teruya-Feldstein J, et al. Use of CD23 (BU38) on paraffin sections in the diagnosis of small lymphocytic lymphoma and mantle cell lymphoma. *Mod Pathol*. 1996;9:925–929.
- Singh N, Wright DH. The value of immunohistochemistry on paraffin embedded tissue sections in the differentiation of small lymphocytic and mantle cell lymphomas. *J Clin Pathol*. 1997;50:16–21.
- Tworek JA, Singleton TP, Schnitzer B, et al. Flow cytometric and immunohistochemical analysis of small lymphocytic lymphoma, mantle cell lymphoma, and plasmacytoid small lymphocytic lymphoma. *Am J Clin Pathol*. 1998;110: 582–589.
- Peghini PE, Fehr J. Analysis of cyclin D1 expression by quantitative real-time reverse transcription-polymerase chain reaction in the diagnosis of mantle cell lymphoma. *Am J Clin Pathol*. 2002;117:237–245.
- Gong JZ, Lagoo AS, Peters D, et al. Value of CD23 determination by flow cytometry in differentiating mantle cell lymphoma from chronic lymphocytic leukemia. *Am J Clin Pathol*. 2002;116:893–897.
- Dunphy CH, Wheaton SE, Perkins SL. CD23 expression in transformed small lymphocytic lymphomas/chronic lymphocytic leukemias and blastic transformations of mantle cell lymphoma. *Mod Pathol*. 1997;10:818–822.
- Dorfman DM, Shahsafaei A. Usefulness of a new CD5 antibody for the diagnosis of T-cell and B-cell lymphoproliferative disorders in paraffin sections. *Mod Pathol*. 1997;10:859–863.



30. Huang JC, Finn WG, Goolsby CL, et al. CD5—small B-cell leukemias are rarely classifiable as chronic lymphocytic leukemia. *Am J Clin Pathol*. 1999;111:123–130.
31. Kamihira S, Hirakata Y, Atogami S, et al. CD5-expressing B-cell lymphomas/leukemias: relatively high frequency of CD5+ B-cell lymphoma with an overall poor prognosis in Nagasaki Japan. *Leuk Lymphoma*. 1996;22:137–142.
32. Matolesy A, Chadburn A, Knowles DM. De novo CD5-positive and Richter's syndrome-associated diffuse large B-cell lymphomas are genotypically distinct. *Am J Pathol*. 1995;147:207–216.
33. Yumaguchi M, Seto M, Okamoto M, et al. De novo CD5+ diffuse large B-cell lymphoma: a clinicopathologic study of 109 patients. *Blood*. 2002;99:815–821.
34. Espinet B, Larriba I, Salido M, et al. Genetic characterization of the paraimmunoblastic variant of small lymphocytic lymphoma/chronic lymphocytic leukemia: a case report and review of the literature. *Hum Pathol*. 2002;33:1145–1148.
35. Picker LJ, Weiss LM, Medeiros LJ, et al. Immunophenotypic criteria for the diagnosis of non-Hodgkin's lymphoma. *Am J Clin Pathol*. 1987;128:181–201.
36. Edelman M, Evans L, Zee S, et al. Splenic micro-anatomical localization of small lymphocytic lymphoma/chronic lymphocytic leukemia using a novel combined silver nitrate and immunoperoxidase technique. *Am J Surg Pathol*. 1997;21:445–452.
37. Sun T, Nordberg ML, Cotelingam JD, et al. Fluorescence in situ hybridization: method of choice for a definitive diagnosis of mantle cell lymphoma. *Am J Hematol*. 2003;74:78–84.
38. Sun T, Susin M, Desner M, et al. The clonal origin of two cell populations in Richter's syndrome. *Hum Pathol*. 1990;21:722–728.
39. Bessudo A, Kipps TJ. Origin of high-grade lymphomas in Richter syndrome. *Leuk Lymphoma*. 1995;18:367–372.
40. Schots R, Dehou MF, Hochmans K, et al. Southern blot analysis in a case of Richter's syndrome. Evidence for a post-rearrangement heavy chain gene deletion associated with the altered phenotype. *Am J Clin Pathol*. 1991;95:571–577.
41. Siegelman MH, Cleary ML, Warnke R, et al. Frequent biclonality and Ig gene alterations among B-cell lymphomas that show multiple histologic forms. *J Exp Med*. 1985;161:850–863.
42. Cleary ML, Galili N, Trela M, et al. Single cell origin of bigenotypic and biphenotypic B-cell proliferations in human follicular lymphomas. *J Exp Med*. 1988;167:582–597.
43. Hamblin TJ, Orchard JA, Ibbotson RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variable in chronic lymphocytic leukemia but CD38 expression may vary during the course of the disease. *Blood*. 2002;99:1023–1029.
44. Gaidano G, Pastore C, Capello D, et al. Molecular pathways in low grade B-cell lymphoma. *Leuk Lymphoma*. 1997;26(suppl 1):107–113.
45. Offit K, Louie DC, Parsa NC, et al. Clinical and morphologic features of B-cell lymphocytic lymphoma with del(6)(q21q23). *Blood*. 1994;83:2611–2618.
46. Ben-Ezra JM, King BE, Harris AC, et al. Staining for bcl-2 protein helps to distinguish benign from malignant lymphoid aggregates in bone marrow biopsies. *Mod Pathol*. 1994;7:560–564.
47. Ligler FS, Smith RG, Keltman JR, et al. Detection of tumor cells in the peripheral blood of non-leukemia patients with B-cell lymphoma. Analysis of "clonal excess." *Blood*. 1980;55:792–800.
48. Drexler HG, Borkhardt A, Janssen JW. Detection of chromosomal translocations in leukemia-lymphoma cells by polymerase chain reaction. *Leuk Lymphoma*. 1995;19:359–380.
49. Thieblemont C, Nasser V, Felman P, et al. Small lymphocytic lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma exhibit distinct gene-expression profiles allowing molecular diagnosis. *Blood*. 2004;103:2727–2737.
50. Schmechel SC, LeVasseur RJ, Yang KHJ, et al. Identification of genes whose expression patterns differ in benign lymphoid tissue and follicular, mantle cell, and small lymphocytic lymphoma. *Leukemia*. 2004;18:841–855.
51. Nola M, Pavletic SZ, Weisenburger DD, et al. Prognostic factors influencing survival in patients with B-cell small lymphocytic lymphoma. *Am J Hematol*. 2004;77:31–35.
52. Kyasa MJ, Hazlett L, Parrish RS, et al. Veterans with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) have a markedly increased rate of second malignancy, which is the most common cause of death. *Leuk Lymphoma*. 2004;45:507–513.

CASE 22

Prolymphocytic Leukemia

CASE HISTORY

A 76-year-old man was found to have lymphocytosis during his hospitalization for the treatment of hypertension and congestive heart failure. His total leukocyte count at that time was 51,000/mL with 76% lymphocytes, 18% neutrophils, and 4% monocytes. His hematocrit was 22% and platelet count 420,000/mL. Investigation of his anemia showed that he had both iron and folate deficiencies; he was treated accordingly. Flow cytometric analysis showed that the immunophenotype of the lymphocytes was consistent with chronic lymphocytic leukemia (CLL). The patient had no hepatosplenomegaly or lymphadenopathy. It was decided at his admission that the patient should be followed for his CLL by oncology service but immediate treatment was not necessary.

In the subsequent 5 years, the patient's leukocyte count fluctuated between 40,000 to 60,000/mL, but finally

reached 150,000/mL with a hematocrit of 20% and platelet count 110,000/mL. The peripheral blood smear revealed 60% prolymphocytes. Physical examination at that time showed splenomegaly. He was treated with chemotherapy, and his total leukocyte count fell to 23,000/mL. However, his absolute neutrophil count dropped to 300/mL. Subsequently, the patient developed *Pneumocystis carinii* pneumonia and enterococcus bacteremia. Despite multi-antibiotic therapy, the patient's condition deteriorated and he died approximately 6 years after the initial diagnosis of CLL.

FLOW CYTOMETRIC FINDINGS

CD5 81%, CD10 2%, CD19 89%, CD19/CD5 80%, CD20 88%, CD23 21%, k 0%, l 93%, FMC-7 82% (Fig. 6.22.1).

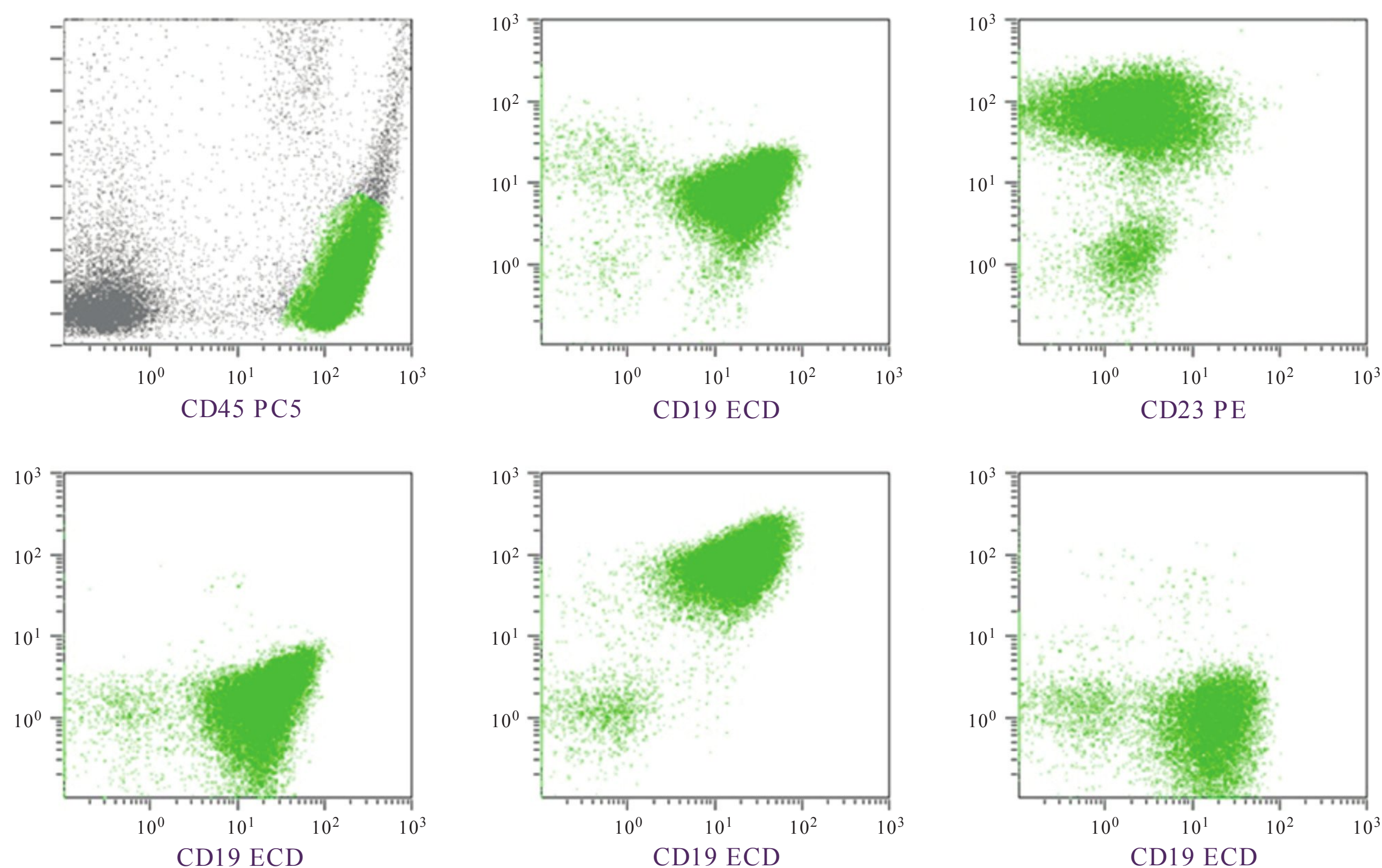


FIGURE 6.22.1 Flow cytometric histograms show dual staining of CD5/CD19, positive FMC-7, partial positive CD23, and monoclonal k pattern. CD10 and l are negative. SS, side scatter; PC5, phycoerythrin-cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ECD, phycoerythrin-Texas Red.

DISCUSSION

Prolymphocytic leukemia (PLL) can be de novo or secondary to CLL (prolymphocytoid transformation). In the case of transformation from CLL, the peripheral blood may show an increase in the number of both prolymphocytes and small lymphocytes, peripheral lymphadenopathy is frequently present, and the leukocyte counts are not as high as in the de novo cases (1–3). Mouse rosette formation with the tumor cells is characteristic for secondary PLL, but this test is no longer a routine test in clinical laboratories. However, in a terminal case when the prolymphocytes become overwhelming, it is difficult to distinguish a primary from a secondary PLL. The definition of PLL is that >55% of the lymphoid cells in the peripheral blood are prolymphocytes (4–6). If the prolymphocyte count is between 10% and 55%, this condition is termed CLL/PLL. A prolymphocyte count <10% is not infrequently seen in a typical CLL case, so that it does not change the diagnosis. The comparison between CLL, CLL/PLL, and PLL is tabulated in Case 19. In the World Health Organization (WHO) classification, secondary PLL is not included as PLL (5–8).

Morphology

A characteristic prolymphocyte of B-cell lineage is about 10 to 15 mm in diameter with a moderate amount of light basophilic cytoplasm (Fig. 6.22.2). The nucleus has a chromatin density between that of a small lymphocyte and that of a lymphoblast. A single prominent nucleolus is the hallmark of a prolymphocyte (Table 6.22.1). In PLL of T-cell lineage, there are two morphologic variants (6,9,10). The small-cell variant is seen in about 25% of cases. The nucleoli are inconspicuous in those cases that require electron microscopic examination to identify the nucleoli (9). In 5% of T-cell PLL (T-PLL) cases, cerebriform nuclei are demonstrated (Sézary cell–like variant) (6). However, those cases

TABLE 6.22.1

Characteristic Morphologic Features of PLL

Histologic pattern	Diffuse infiltration with or without proliferation centers in lymph nodes; bizonal pattern in white pulp of spleen
Cytology	Chromatin density between lymphoblasts and mature lymphocytes with a single prominent nucleolus and moderate amount of cytoplasm
Specific features	Presence of exclusively immature cells without a high mitotic rate

with small lymphoid cells and inconspicuous nucleoli are considered T-cell CLL by some authors (11,12).

Patients with PLL usually show extremely high leukocyte count in the peripheral blood (>100,000/mL) (5) and extensive leukemic infiltration in virtually every organ (13). In the spleen, both the red and the white pulp are infiltrated. A proliferative nodule with a bizonal appearance (darker at the center and lighter at the periphery) in the white pulp or an inverse pseudofollicular pattern is characteristic of PLL (Figs. 6.22.3 and 6.22.4) (13–15). The cells in the center are mature lymphocytes, which are encircled by a rim of prolymphocytes that stain lighter because of their dispersed chromatin. However, in one study of T-PLL cases, the more prominent feature is the expansion of the red pulp with intrasinusoidal lymphoid infiltration (16).

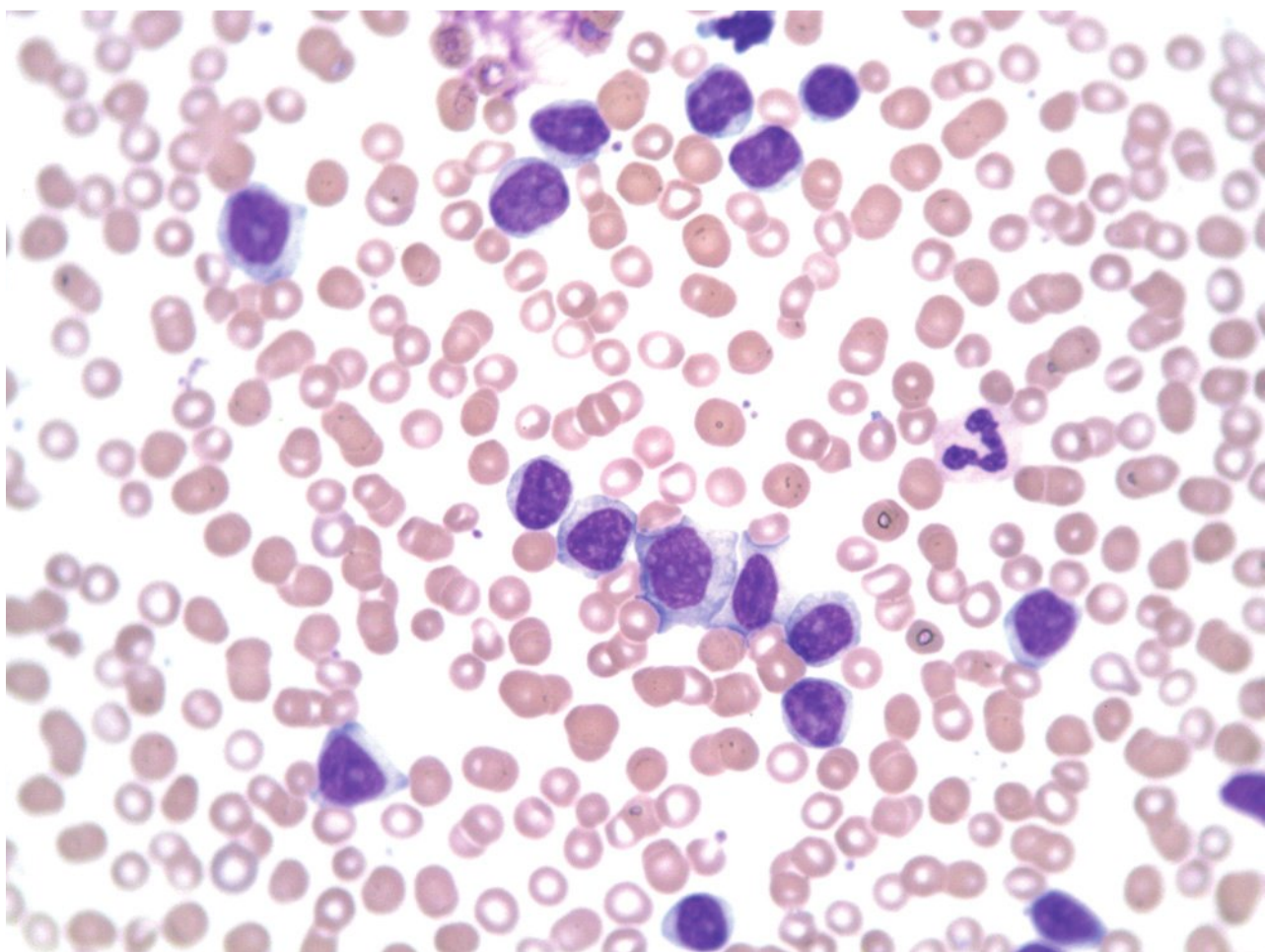


FIGURE 6.22.2 Peripheral blood smear reveals several prolymphocytes with single prominent nucleolus and moderate amount of cytoplasm. Wright–Giemsa, 60× magnification.

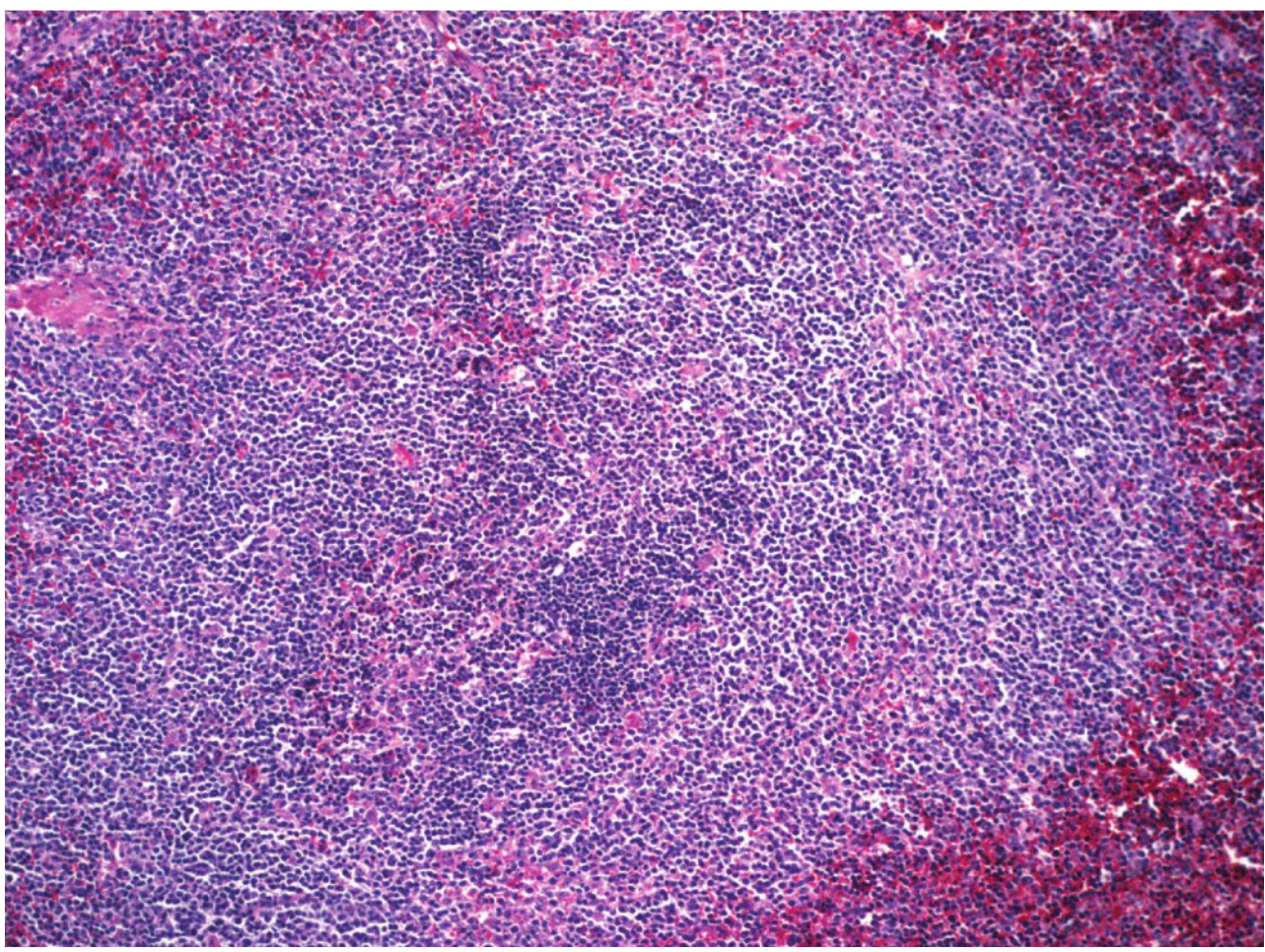


FIGURE 6.22.3 Splenectomy specimen shows a lymphoid follicle with a bizonal pattern (a dense small lymphocyte population in the center and the larger prolymphocytes in the periphery). Hematoxylin and eosin, 10× magnification.

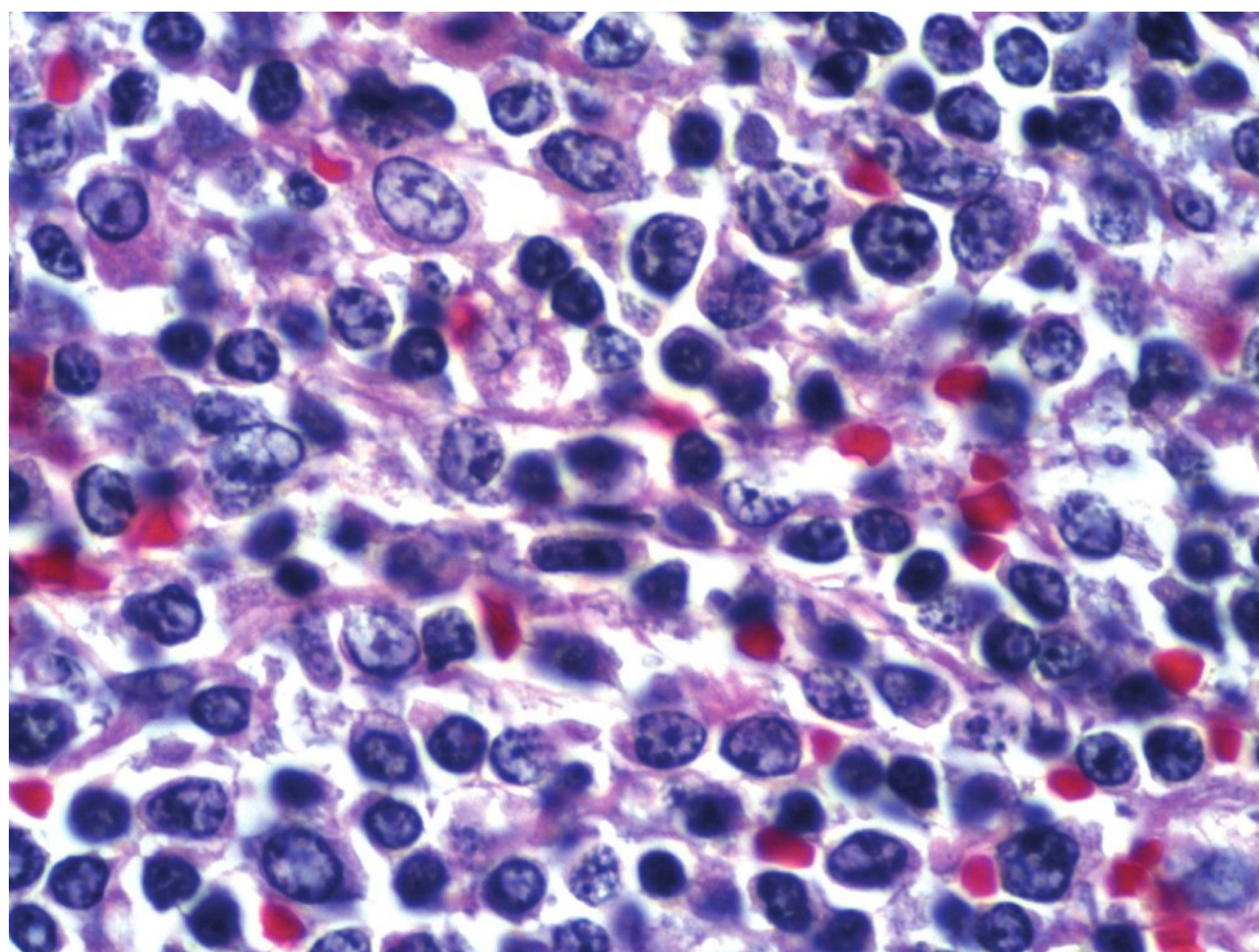


FIGURE 6.22.4 A high-power view of the previous spleen section shows the large prolymphocytes with immature chromatin pattern. Hematoxylin and eosin, 100× magnification.

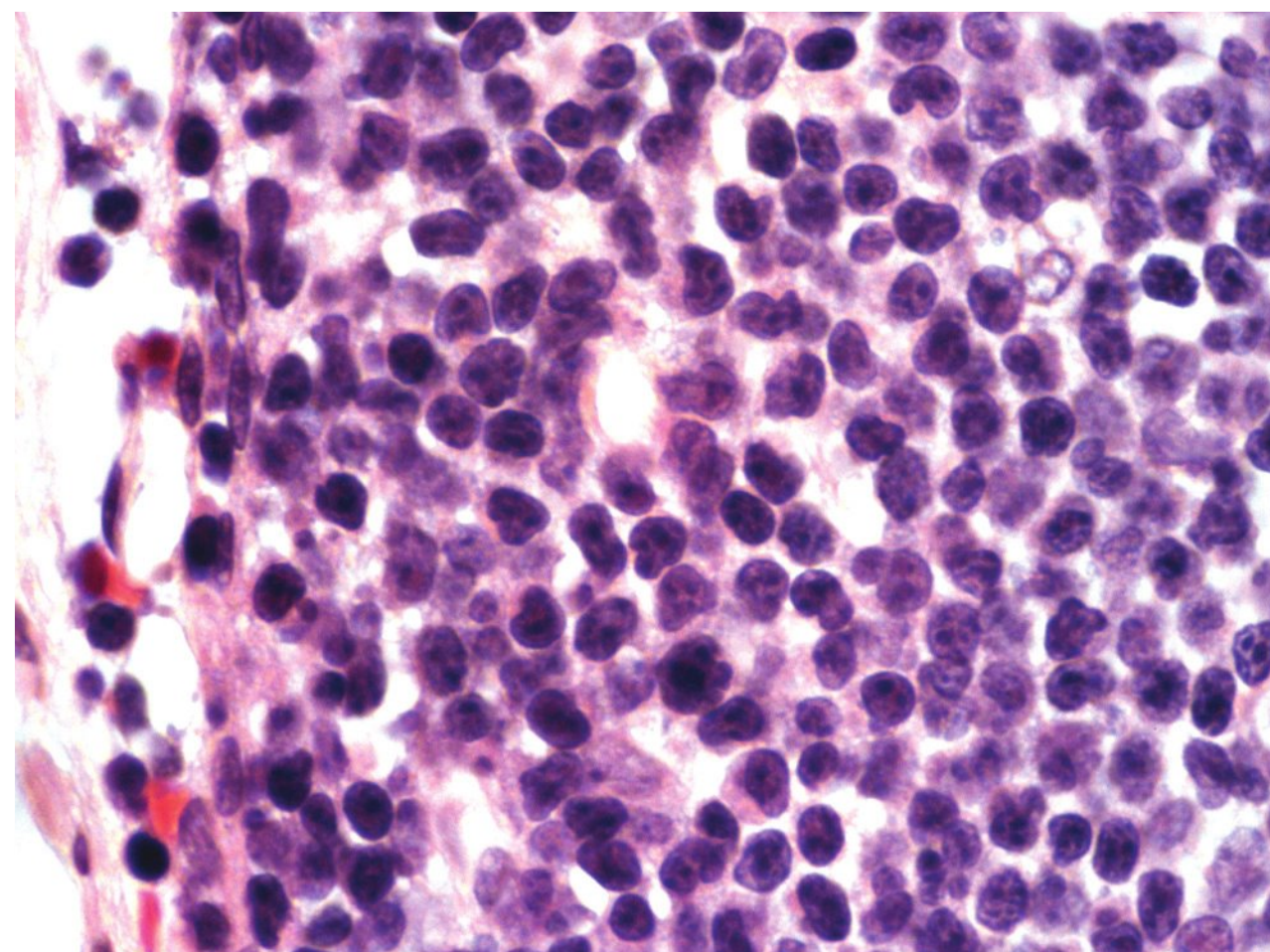


FIGURE 6.22.6 A high-power view of the previous lymph node section shows the immature-looking prolymphocytes. The absence of mitosis and apoptosis among the immature cells is characteristic of prolymphocytoid transformation. Hematoxylin and eosin, 100× magnification.

The lymph node, if involved, shows a diffuse infiltration with or without a pseudonodular pattern (13,14). One study of three lymph nodes showed a paracortical distribution of the tumor cells (3). In PLL secondary to CLL, the infiltration may be patchy. When the normal architecture is completely replaced by prolymphocytes and paraimmunoblasts (Figs. 6.22.5 and 6.22.6), it is frequently referred to as a paraimmunoblast variant of small lymphocytic lymphoma/leukemia (14,17). Paraimmunoblasts are cells larger than prolymphocytes with very prominent nucleoli and abundant cytoplasm, and, in some cases, there are only a few of these cells present among the prolymphocytes in the lymph node with this diagnosis (14). Therefore, the term prolymphocytoid paraimmunoblastic transformation used by

Brunning and McKenna (14) is probably more appropriate if prolymphocyte is predominant.

The bone marrow infiltration is interstitial or diffuse, similar to that seen in CLL, or shows a mixed interstitial-nodular pattern (Figs. 6.22.7 and 6.22.8) (3,13,14). In the skin, the characteristic of T-PLL is perivascular and peri-appendiceal infiltration by atypical lymphoid cells in the dermis without epidermotropism (18,19).

The prolymphocytes in tissue sections appear medium to large in size with a distinct rim of cytoplasm and a round to oval nucleus. The nuclear chromatin pattern is dispersed, and a single variably prominent nucleolus is usually present (14). Despite the immature appearance of the tumor cells, the mitotic rate is usually low; this combination is considered characteristic of PLL (13).

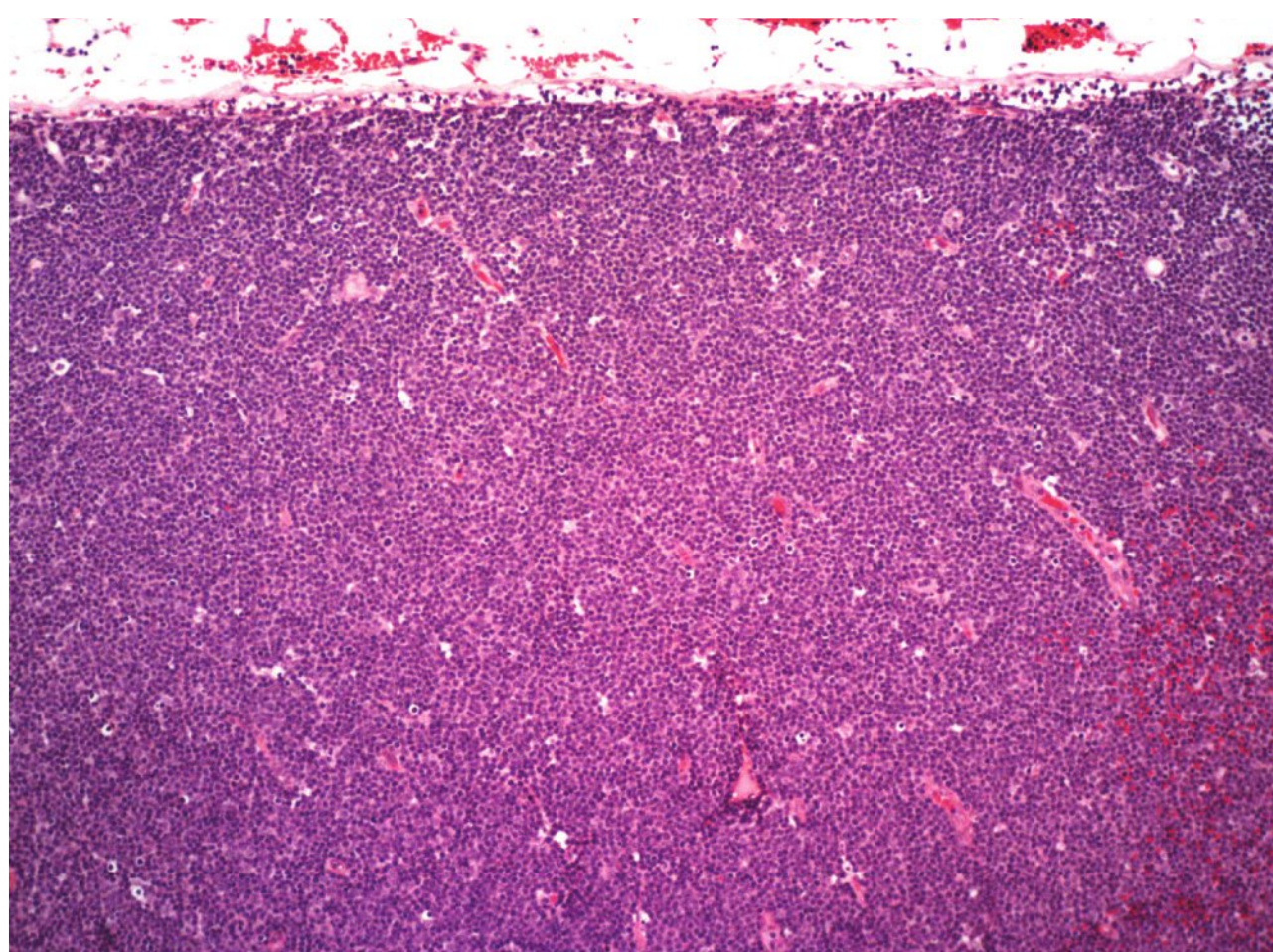


FIGURE 6.22.5 Lymph node biopsy reveals effacement of the normal architecture by a monotonous population of prolymphocytes. Hematoxylin and eosin, 20× magnification.

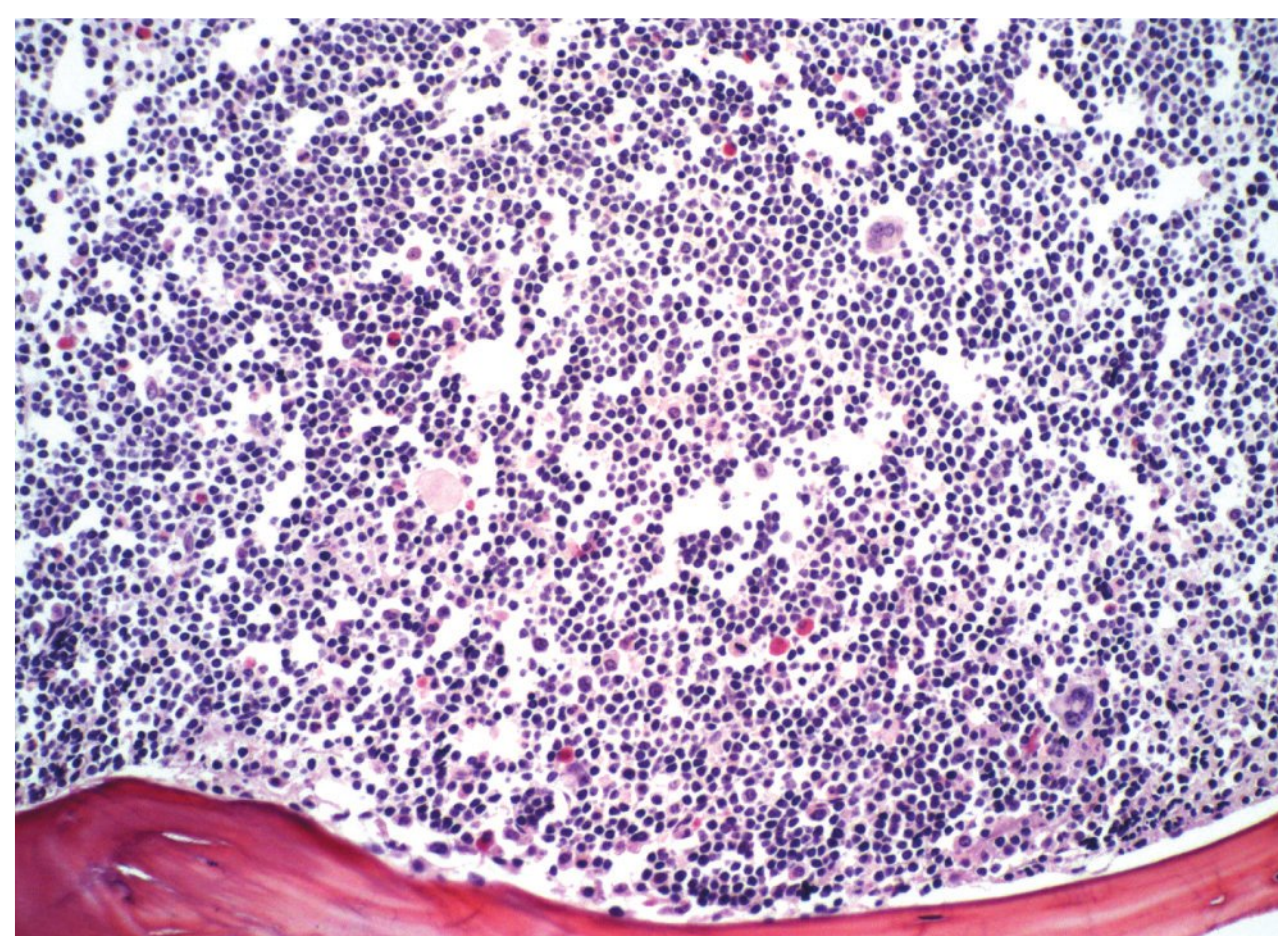


FIGURE 6.22.7 Bone marrow core biopsy shows diffuse prolymphocytic infiltration. Hematoxylin and eosin, 20× magnification.

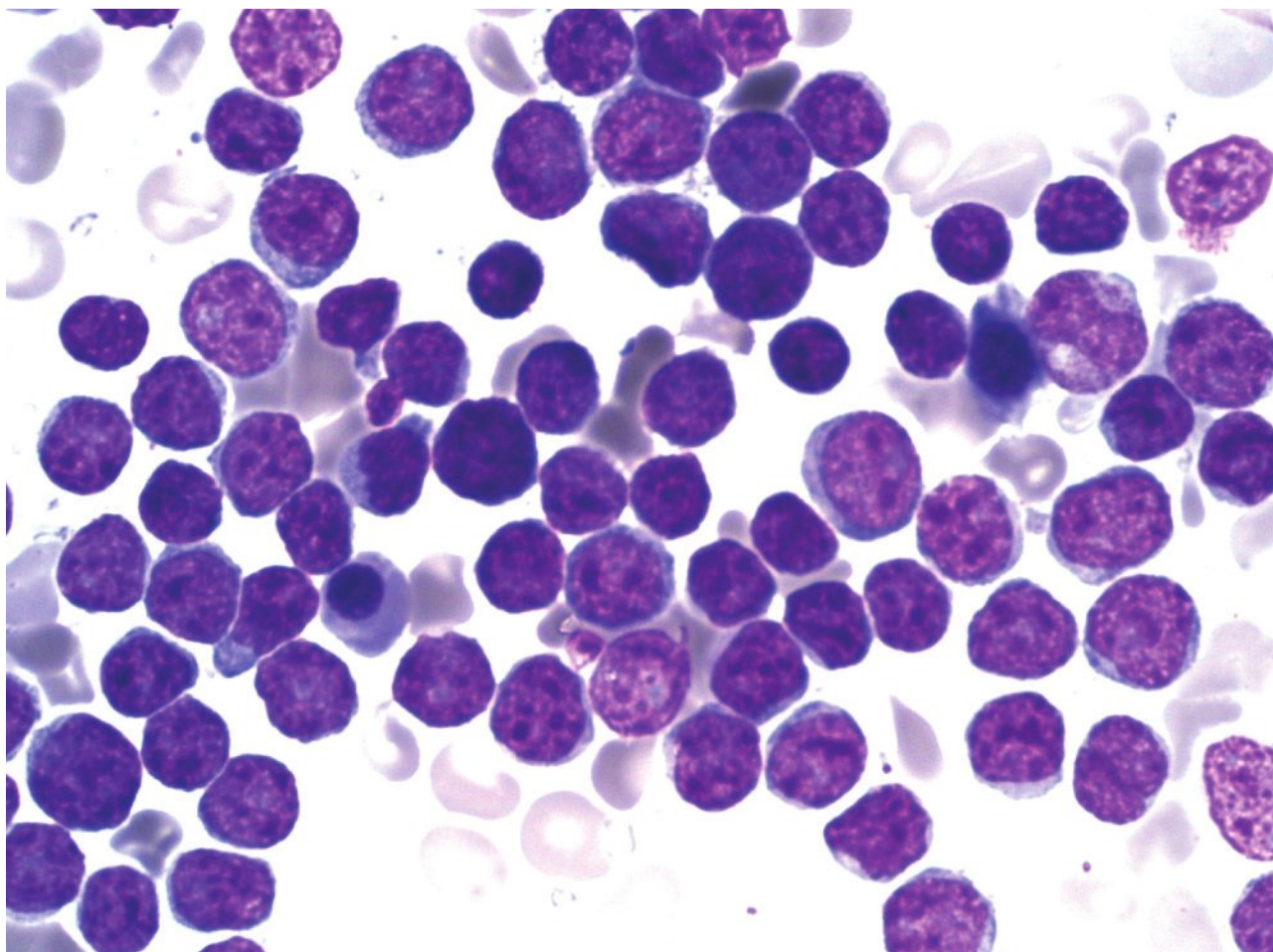


FIGURE 6.22.8 Bone marrow aspirate reveals a pure population of prolymphocytes. The prolymphocytes in the bone marrow usually do not show moderate amounts of cytoplasm. Wright–Giemsa, 100× magnification.

Immunophenotype

Although typical CLL cells and PLL cells are quite different morphologically, many “transitional” cells with an appearance between those of CLL and PLL, as seen in prolymphocytoid transformation, are not easy to distinguish. Immunophenotyping is helpful in this situation as well as in distinguishing primary and secondary PLL.

Prolymphocytes usually show a bright staining of the surface immunoglobulin, whereas CLL cells typically show dim immunofluorescence. Despite the difference in the density of surface immunoglobulin, both CLL and PLL patients have hypogammaglobulinemia, probably related to imbalance in T-cell subsets (20). In contrast, monoclonal gammopathy is much more frequently seen in PLL than in CLL patients (21).

Another marker that distinguishes between CLL and PLL is the presence of mouse rosetting in CLL cells, which is absent in most PLL cells, except for some cases of secondary PLL (4,14,21). This phenomenon, in addition to the higher density of surface immunoglobulin on PLL cells, indicates that PLL cells are derived from a more mature progenitor than CLL cells are, yet PLL is clinically more aggressive and more refractory to therapy, resulting in a shorter survival for PLL than for CLL patients (22).

Three monoclonal antibodies (CD5, FMC-7, and CD22) are particularly helpful in distinguishing CLL from PLL. CD5 is positive for CLL but is negative for two thirds of PLL cases (21). However, if PLL is transformed from CLL, CD5 may remain positive (14). FMC-7 and CD22 are positive for PLL but negative for most CLL cases. The presence of FMC-7 is particularly important in the diagnosis of PLL (23). CD19, CD20, CD23, and HLA-DR are positive for both (3,22,24).

Moderate expression of CD79b in PLL has been reported (25). The important negative markers for PLL are terminal deoxynucleotidyl transferase (TdT) and CD10, which help to distinguish PLL from lymphoblastic lymphoma/

leukemia (4,22). PLL cells are also negative for the terminal B-cell antigens such as plasma cell–associated antigen (PCA-1) and CD38 (4,22,24). Negative CD25 in PLL distinguishes it from hairy cell leukemia, which is positive for CD25 (4,19,20). However, PLL can be induced by phorbol ester into hairy cell leukemia (26), and a hybrid form of hairy cell leukemia and PLL has been reported (27,28).

About 20% of PLL cases are of the T-cell type (29). The largest reported series of T-PLL included 78 patients studied over 12 years (9). In this series, membrane marker studies defined a postthymic phenotype (TdT–, CD2+, CD3+, CD5+, CD7+) for the tumor cells. Among them, 55% to 65% were CD4+, CD8– 21% to 30% CD4+, CD8+, and 10% to 13% CD4–, CD8+ (9,30).

One important antigen that identifies malignant cells of T-PLL is CD52, because patients showing this marker can be treated with alemtuzumab (anti-CD52) with promising results (31). T-cell leukemia-1 protein (TCL1), the product of TCL1 oncogene, is overexpressed in T-PLL and can be demonstrated by immunohistochemistry (32). It is useful in distinguishing other T-cell neoplasms, but TCL1 has also been reported in many B-cell lymphomas (19).

Comparison between Flow Cytometry and Immunohistochemistry

Flow cytometry is more helpful than immunohistochemistry in differential diagnosis between various lymphomas that are similar to B-cell PLL (B-PLL). A high percentage of FMC-7 is particularly useful for the diagnosis of PLL, despite the fact that this marker is also present in hairy cell leukemia, mantle cell lymphoma, and various marginal zone lymphomas. However, immunohistochemistry is most helpful in identifying T-PLL by morphologic correlation.

Molecular Genetics

In the Mittleman catalog (33), the most common cytogenetic abnormalities in B-PLL involve chromosomes 1, 3, 6, 8, and 11 to 14. The most common cytogenetic aberrations include t(11;14)(q13;q32), t(14;17)(q32;q11), t(6;12)(q15;p13), del(3)(p13), and trisomy 12 (33). In a study of nine B-PLL cases, 60% showed 14q+ abnormality (34). Another study of 20 patients revealed monosomy 17 or additions to 17p in 45% of cases (3). 17p deletions involving the TP53 gene, and rearrangements of the MYC oncogene at 8q24 are seen in approximately 50% of B-PLL cases in some studies (35). Trisomy 12 is more frequently demonstrated in patients with secondary PLL than de novo PLL, as it is the most common cytogenetic abnormality in CLL cases (3). Deletions at 11q23 and 13q14 have been found to be frequent abnormalities in B-PLL by the fluorescence in situ hybridization technique (36).

Recently, several authors have advocated reclassification of PLL with t(11;14) as mantle cell lymphoma, because those cases usually have the immunophenotype of mantle cell lymphoma (CD5+, CD23–, cyclin D1+) and are clinically very aggressive (3,37). Because the B-PLL may have cytogenetic abnormalities (e.g., 17q) that are present in other low-grade lymphoproliferative disorders, B-cell leukemia with prolymphocytic morphology may represent a common end stage of transformation for several B-cell neoplasms (3).

In T-PLL cases, chromosome 14 inversion or translocation with breakpoints at bands q11 and q32 are the most frequent findings (10,30,38). In 90% of T-PLL cases, the TCL1 and TCL1b genes at 14q32.1 or the MTCPI gene at Xq28 are involved in translocations or inversions with TCRα at 14q11 (38).

The second common cytogenetic abnormality involves chromosome 8, including i(8)(q10), t(8;8)(p21;q11), +8, and 8p+ (10,30,39). Other rare aberrations include del(6q), del(11q), -17, and -22 (28). It is of interest that patients having ataxia telangiectasis with T-cell clones in their blood carrying 14q11 and 14q32 chromosomal rearrangements are at risk of developing T-cell malignancies, including T-PLL (7,40,41).

B-PLL usually shows immunoglobulin heavy chain gene rearrangement with preferential VH3 and VH4 family gene segment usage (7,42). In T-PLL, TCRβ and TCRγ genes are clonally rearranged (38).

Prediction analysis for microarray (PAM) identified 46 genes that helped to differentiate B-PLL and CLL (43). In a microarray experiment, it was concluded that the overexpression of C-MYC and AKT combined with TP53 impairment might have a central role in the pathogenesis of B-PLL, promoting apoptosis inhibition and cell proliferation (43). A molecular allelkaryotyping of T-PLL revealed multiple aberrations, involving chromosomes 8, 11, and 14 (44). New recurrent alterations were identified on chromosome 5p, 12p, 13q, 17, and 22 (44).

In the current case, the patient had a long history of CLL, but he was not treated until he showed signs of prolymphocytoid transformation—namely, leukocytosis >150,000/mL with 60% prolymphocytes in the peripheral blood. His hematocrit and platelet count also dropped precipitously. Splenomegaly became prominent. His immunophenotype was also characteristic of secondary PLL by showing a high percentage of dual CD19/CD5-positive cells. Although he responded to chemotherapy with decreased leukocytosis, he also became leukopenic with leukopenic fever. The subsequent development of opportunistic infections led to his fatal outcome. He died approximately 1 year after prolymphocytoid transformation.

The salient features for laboratory diagnosis of PLL are summarized in Table 6.22.2.

Clinical Manifestations

PLL is seen about 10% as frequently as CLL, and the median age of PLL patients is 70 years compared with 64 years for those with CLL (22,45). Massive splenomegaly is characteristic of PLL, but hepatomegaly and lymphadenopathy are usually absent or mild in B-PLL (5). In T-PLL, however, lymphadenopathy is present in 26% to 53% of patients, hepatomegaly in 40% to 55%, and skin infiltration in 25% to 27% (9,46). Leukemic meningitis has been reported in a few cases of PLL or CLL/PLL (47). Meningitis may occur in early and advanced stages of the leukemia. Intrathecal chemotherapy alone is effective in clearing up the leukemic cells in the cerebrospinal fluid and eliminating neurologic symptoms caused by leukemic infiltrates (47). At the time of diagnosis, most patients have advanced-stage disease (i.e., Rai Stage IV or Benet Stage C) (48).

TABLE 6.22.2

Salient Features for Laboratory Diagnosis of PLL

1. Total leukocyte count > 100,000/μL
2. Presence of >55% of prolymphocytes in peripheral blood lymphoid population
3. FCM shows cells in large lymphocyte gate with bright fluorescence.
4. Monoclonal surface immunoglobulin, usually IgM-κ
5. Immunophenotype for B-cell PLL: Positive for CD19, CD20, CD22, FMC-7, HLA-DR; negative for TdT, CD10, and CD5 (2/3 cases)
6. Immunophenotype for T-cell PLL: TdT-, CD2+, CD3+, CD5+, CD7+; 55%–65% CD4+ CD8-, 21%–30% CD4+ CD8+, and 10%–13% CD4- CD8+
7. Immunoglobulin gene rearrangements in B-cell PLL
8. T-cell receptor gene rearrangements in T-cell PLL
9. Cytogenetic abnormalities: 17p abnormalities and 14q+ are frequent for B-cell PLL, inv(14)(q11q32), t(14;14)(q11;q32), and chromosome 8 abnormalities are frequent for T-cell PLL.
10. TCL1 and TCL1b genes at 14q32.1 and TCRα at 14q11 are involved in the inv(14) and t(14;14).

FCM, flow cytometry; PLL, prolymphocytic leukemia; TdT, terminal deoxynucleotidyl transferase.

A definitive diagnosis is based on the extremely high leukocyte counts, >100,000/mL in about 75% of patients, and the lymphoid cell should be composed of >55% prolymphocytes (4). The median survival in B-PLL is 2 to 3 years compared with 8 years in CLL (49). Patients with PLL of T-cell origin have a median survival of 7 to 7.5 months (9,30) and rarely survive 2 years after diagnosis (50).

REFERENCES

1. Kjeldberg CR, Marry J. Prolymphocytic transformation of chronic lymphocytic leukemia. *Cancer*. 1981;48:2447–2457.
2. Ghani AM, Krause JR, Brody JP. Prolymphocytic transformation of chronic lymphocytic leukemia: a report of three cases and review of the literature. *Cancer*. 1986;57:75–80.
3. Schlette E, Bueso-Ramos C, Giles F, et al. Mature B-cell leukemia with more than 55% prolymphocytes: a heterogeneous group that includes an unusual variant of mantle cell lymphoma. *Am J Clin Pathol*. 2001;115:571–581.
4. Bennet JM, Catovsky D, Daneil MT, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukemias. *J Clin Pathol*. 1989;42:567–584.
5. Catovsky D, Montserrat E, Müller-Hermelink HK, et al. B-cell prolymphocytic leukaemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:131–132.
6. Catovsky D, Ralfkiaer E, Müller-Heermelink HK. T-cell prolymphocytic leukemia. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:195–196.

7. Campo E, Catovsky D, Montserrat E, et al. B-cell prolymphocytic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al. eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:183–184.
8. Catovsky D, Müller-Hermelink HK, Ralfkiaer E. T-cell prolymphocytic leukemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th ed. Lyon, France: IARC Press; 2008:270–271.
9. Matutes E, Brito-Babapulle V, Swansbury J, et al. Clinical and laboratory features of 78 cases of T-prolymphocytic leukemia. *Blood*. 1991;78:3269–3274.
10. Garand R, Goasguen J, Brizard A, et al. Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia. *Br J Haematol*. 1998;103:488–494.
11. Hoyer JD, Ross CW, Li CY, et al. True T-cell chronic lymphocytic leukemia: a morphologic and immunophenotypic study of 25 cases. *Blood*. 1995;86:1163–1169.
12. Matutes E, Catovsky D. Similarity between T-cell chronic lymphocytic leukemia and the small cell variant of T-prolymphocytic leukemia. *Blood*. 1996;87:3520–3521.
13. Bearman RM, Pangalis GA, Rappaport H. Prolymphocytic leukemia: clinical histopathological and cytochemical observations. *Cancer*. 1989;42:2360–2372.
14. Brunning RD, McKenna RW. Tumor of the Bone Marrow. Washington, DC: Armed Forces Institute of Pathology; 1994:266–301.
15. Lampert IA, Thompson I. The spleen in chronic lymphocytic leukemia and related disorders. In: Pollick A, Catovsky D, eds. Chronic Lymphocytic Leukemia. Chur, Switzerland: Harwood Academic Publishers; 1988:193–208.
16. Osuji N, Matutes E, Catovsky D, et al. Histopathology of the spleen in T-cell large granular lymphocyte leukemia and T-cell prolymphocytic leukemia: a comparative review. *Am J Surg Pathol*. 2005;29:935–941.
17. Pugh WC, Manning JT, Butler JJ. Paraimmunoblastic variant of small lymphocytic lymphoma/leukemia. *Am J Surg Pathol*. 1988;12:907–917.
18. Mallet RB, Matutes E, Catovsky D, et al. Cutaneous infiltration in T-cell prolymphocytic leukaemia. *Br J Dermatol*. 1995;132:263–266.
19. Valbuena JR, Herling M, Admirand JH, et al. T-cell prolymphocytic leukemia involving extramedullary sites. *Am J Clin Pathol*. 2005;123:456–464.
20. Caligaris-Cappio F, Janossy G. Surface markers in chronic lymphoid leukemias of B-cell type. *Semin Hematol*. 1985; 22:1–12.
21. Skarin AT. Pathology and morphology of chronic lymphocytic leukemias and related disorders. In: Wiernik PH, Canellos GP, Kyle RA, et al., eds. Neoplastic Diseases of the Blood. New York: Churchill Livingstone; 1985:19–24.
22. Stone RM. Prolymphocytic leukemia. *Hematol Oncol Clin North Am*. 1990;4:457–471.
23. Huh YO, Pugh WC, Kantarjian HM, et al. Detection of subgroup of chronic B-cell leukemia by FMC7 monoclonal antibody. *Am J Clin Pathol*. 1994;101:283–289.
24. Melo JV, Robinson DSF, Catovsky D. The differential diagnosis between chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders: morphological and immunological studies. In: Polliack A, Catovsky D, eds. Chronic Lymphocytic Leukemia. Chur, Switzerland: Harwood Academic Publishers; 1988:85–103.
25. Cabezudo E, Carrara P, Morilla R, et al. Quantitative analysis of CD79b, CD5, and CD19 in mature B-cell lymphoproliferative disorders. *Haematologica*. 1999;84:413–418.
26. Zieger-Heitbrock HW, Munker R, Dorken B, et al. Induction of features characteristic of hairy cell leukemia in chronic lymphocytic leukemia and prolymphocytic leukemia cells. *Cancer Res*. 1986;46:2172–2178.
27. Catovsky D, O'Brien M, Melo JV, et al. Hairy cell leukemia variant: an intermediate disease between HCL and B prolymphocytic leukemia. *Semin Oncol*. 1984;11:362–369.
28. Dunphy CH, Petruska PJ. Atypical prolymphocytic variant of hairy-cell leukemia: case report and review of the literature. *Am J Hematol*. 1996;53:121–125.
29. Catovsky D, Weckslar A, Matutes A, et al. The membrane phenotype of T-prolymphocytic leukemia. *Scand J Haematol*. 1982;29:398–404.
30. Mossfa H, Brizard A, Hutet JL, et al. Trisomy 8q due to i(8q) or der(8), t(8:8) is a frequent lesion in T-prolymphocytic leukaemia: four new cases and a review of the literature. *Br J Haematol*. 1994;86:780–785.
31. Darden CD. T-cell prolymphocytic leukemia. *Med Oncol*. 2006;23:17–22.
32. Pekarsky Y, Hallas C, Croce CM. Molecular basis of mature T-cell leukemia. *JAMA*. 2002;286:2308–2314.
33. Mittleman F. Catalog of Chromosome Aberrations in Cancer. 5th ed. New York: Wiley-Liss; 1994.
34. Pittman S, Catovsky D. Chromosome abnormalities in B-cell prolymphocytic leukemia: a study of nine cases. *Cancer Genet Cytogenet*. 1983;9:355–365.
35. Viswanatha DS, Montgomery KD, Foucar K. Mature B-cell neoplasms: chronic lymphocytic leukemia-small lymphocytic lymphoma, B-cell prolymphocytic leukemia, and lymphoplasmacytic lymphoma. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. Hematopathology. Philadelphia, PA: Elsevier; 2011:221–246.
36. Lens D, Matutes E, Catovsky D, et al. Frequent deletions at 11q23 and 13q14 in B cell prolymphocytic leukemia (B-PLL). *Leukemia*. 2000;14:427–430.
37. Ruchlemer R, Parry-Jones N, Brito-Babapulle V, et al. B-prolymphocytic leukaemia with t(11;14) revisited: a splenomegalic form of mantle cell lymphoma evolving with leukaemia. *Br J Haematol*. 2004;125:330–336.
38. Porwit A, Djokic M. T-cell prolymphocytic leukemia. In: Jaffe ES, Harris NL, Vardiman JW, et al. Hematopathology. Philadelphia, PA: Elsevier; 2011:513–520.
39. Maljaei SH, Brito-Babapulle V, Hiorns LR, et al. Abnormalities of chromosomes 8, 11, 14, and X in T-prolymphocytic leukemia studied by fluorescence in situ hybridization. *Cancer Genet Cytogenet*. 1998;103:110–116.
40. Brito-Babapulle V, Catovsky D. Inversion and random translocation involving chromosome 14q11 and 14q32 in T-prolymphocytic leukemia and T-cell leukemia in patients with ataxia telangiectasia. *Cancer Genet Cytogenet*. 1991; 55:1–9.
41. Luo L, Lu FM, Hart S, et al. Ataxia-telangiectasia and T-cell leukemias: no evidence for somatic ATM mutation in sporadic T-ALL or for hypermethylation of the ATM-NPAT/E14 bidirectional promoter in T-PLL. *Cancer Res*. 1998;58:2293–2297.
42. Melo JV, Foroni L, Brito-Babapulle V, et al. Prolymphocytic leukemia of B-cell type: rearranged immunoglobulin (Ig) genes with defective Ig production. *Blood*. 1985;66:391–398.
43. Del Giudice I, Osuji N, Dexter T, et al. B-cell prolymphocytic leukemia and chronic lymphocytic leukemia have distinctive gene expression signatures. *Leukemia*. 2009;23:2160–2167.
44. Nowak D, Le Toriell E, Stern M-H, et al. Molecular allelo-karyotyping of T-cell prolymphocytic leukemia cells with high density single nucleotide polymorphism arrays identifies novel common genomic lesions and acquired uniparental disomy. *Haematologica*. 2009;94:518–527.

45. Melo JV, Catovsky D, Galton DA. The relationship between chronic lymphocytic leukemia and prolymphocytic leukemia. 1. Clinical and laboratory features of 300 patients and characteristic of an intermediate group. *Br J Haematol*. 1986;63:377–387.
46. Matutes E, Catovsky D. Mature T-cell leukemias and leukemia/lymphoma syndromes: review of our experience in 175 cases. *Leuk Lymphoma*. 1991;4:81–91.
47. Hoffman MA, Valderrama E, Fuchs A, et al. Leukemic meningitis in B-cell lymphocytic leukemia: a clinical, pathologic, and ultrastructural case study and a review of the literature. *Cancer*. 1995;75:1100–1103.
48. Foucar MK. B-cell chronic lymphocytic leukemia and prolymphocytic leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1505–1529.
49. Melo JV, Catovsky D, Gregory WM, et al. The relationship between chronic lymphocytic leukemia and prolymphocytic leukemia. IV. Analysis of survival and prognostic features. *Br J Haematol*. 1987;65:23–29.
50. Markey G, Morris TCM, Alexander D, et al. Prognosis in T prolymphocytic leukemia. *Lancet*. 1986;1:381–382.

CASE 23

Lymphoplasmacytic Lymphoma

CASE HISTORY

A 65-year-old man was admitted to the hospital for workup of enlarged lymph nodes. Three weeks prior to admission, the patient began to notice multiple enlarged lymph nodes, most predominantly in his right axillary region. One week after, he also noticed the onset of night sweats, fevers, and chills. A lymph node biopsy performed in a local hospital was suspicious of lymphoma. He also reported a 40-lb weight loss in the past year.

Physical examination on admission showed that he had lymphadenopathy in the submandibular, cervical, supraclavicular, axillary, and inguinal regions. These lymph nodes measured from 0.2 to 1.5 cm in diameter and were rubbery and mobile, with mild tenderness but no erythema. No hepatosplenomegaly was present.

Hematologic workup revealed a total leukocyte count of 7,300/mL with a normal differential count. His hematocrit was 36%, hemoglobin 12 g/dL, and platelet count 200,000/mL. Due to the presence of hemagglutination, cold agglutinin was tested, which showed a titer of 3,000. Reticulocyte count was 7%, bilirubin 1.4 mg/dL, and lactate dehydrogenase 230 U/L. Immunoglobulin studies revealed IgG 944 mg/dL, IgM 350 mg/dL, IgA 340 mg/dL, k 1300 mg/dL, and l 447 mg/dL. Immunofixation showed a monoclonal IgM-k pattern (Fig. 6.23.1). A lymph node and a bone marrow biopsy were performed.

FLOW CYTOMETRY FINDINGS

Lymph node biopsy: B-cell markers: CD19 57%, CD20 4%, CD23 8%, surface k 75%, surface l 0%. Plasma cell markers: CD38 60%, CD138 10%, CD56 0%, cytoplasmic k 65%, and cytoplasmic l 0%. T-cell marker: CD5 0% (Fig. 6.23.2).

IMMUNOHISTOCHEMICAL STAINS

Lymphocytes revealed predominantly CD20 staining with a small percentage of lymphocytes showing CD3 reaction. The plasma cells were positive for k stain in 90% of cells, and l in 10%.

CYTOGENETIC STUDY

Karyotyping of the bone marrow specimen revealed a normal male karyotype of 46, XY.

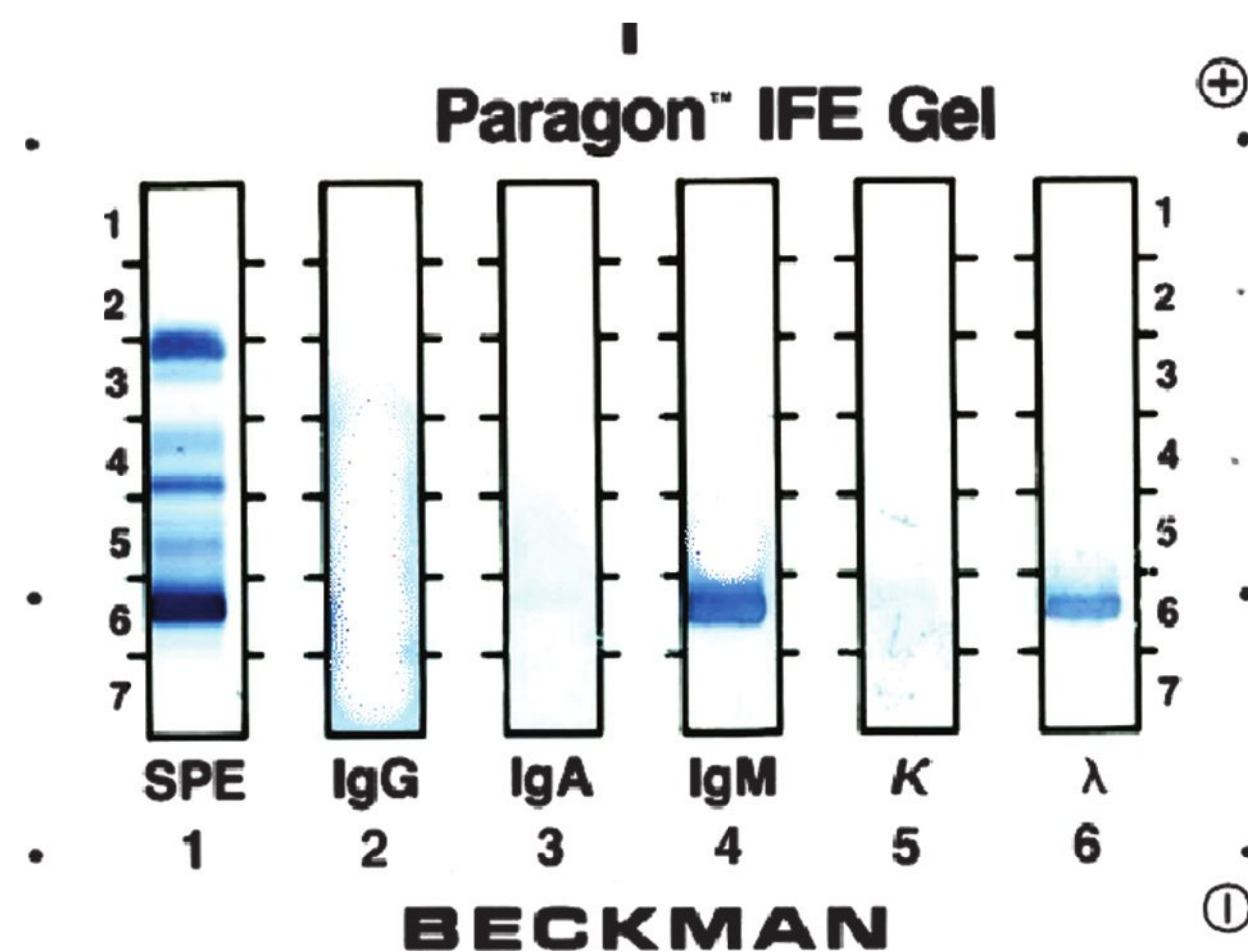


FIGURE 6.23.1 Immunofixation electrophoresis shows a monoclonal IgM-λ pattern.

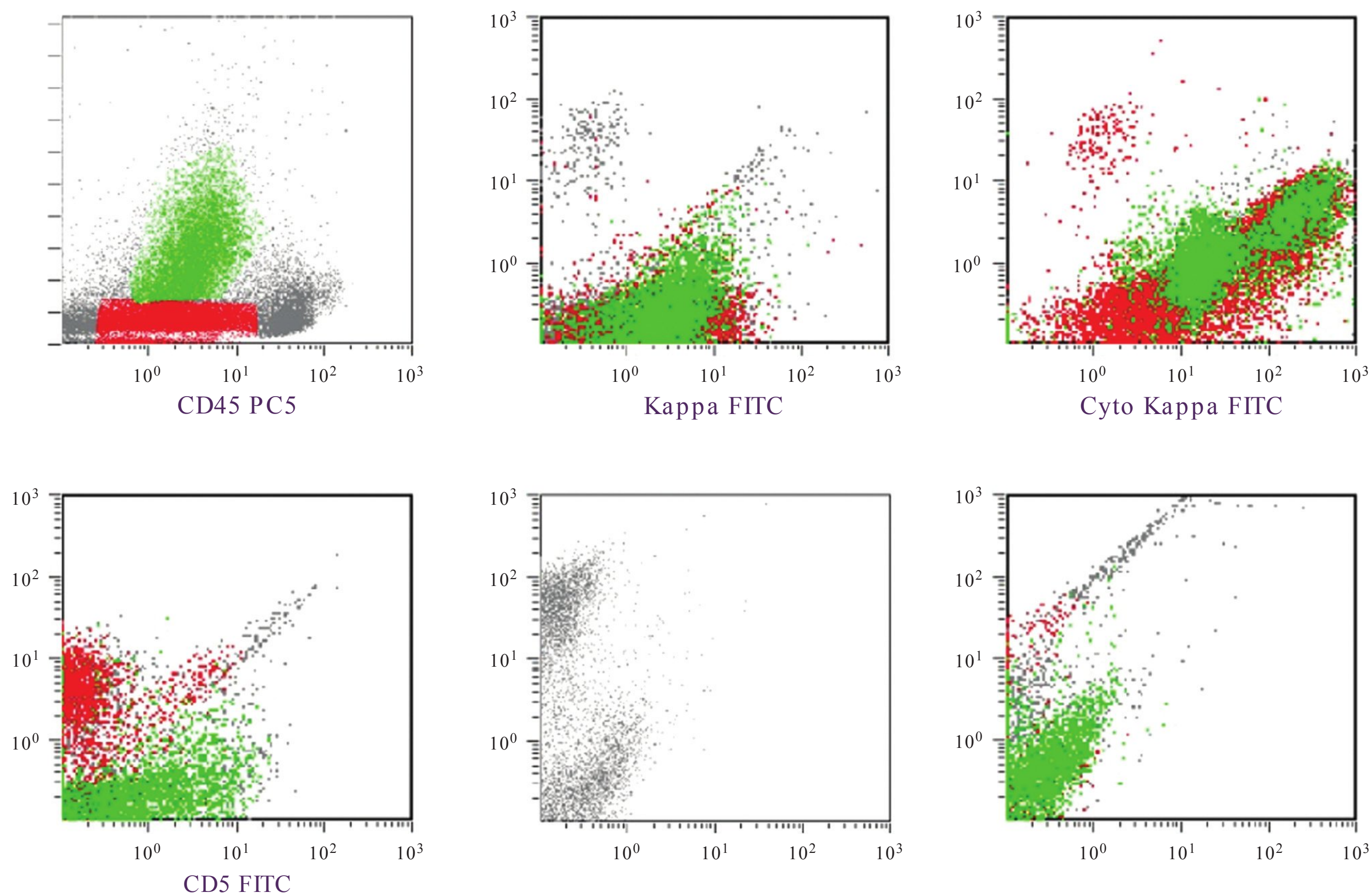


FIGURE 6.23.2 Flow cytometric analysis shows a heterogeneous tumor cell population. The green cluster represents mainly the CD19-negative plasma cell population. The red cluster represents mainly the CD19-positive B lymphocytes. Both populations show a dual monoclonal surface and cytoplasmic k pattern and are negative for CD23 and CD10. The uncolored cluster represents the normal lymphocyte population. SS, side scatter; PC5, phycoerythrin-cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

DISCUSSION

In the 2001 World Health Organization (WHO) classification, lymphoplasmacytic lymphoma (LPL) and Waldenström macroglobulinemia (WM) are considered synonymous (1). This combined entity is defined as “a neoplasm of small B lymphocytes, plasmacytoid and plasma cells, usually involving bone marrow, lymph nodes and spleen, usually lacking CD5, which has a serum monoclonal protein with hyperviscosity or cryoglobulinemia in most cases” (1). However, the diagnostic criteria are still controversial (2–4), and recent cytogenetic evidence appears to indicate that WM is only associated with a subtype of LPL (5,6). LPL cases with t(9;14) are seldom, if ever, associated with WM. The 2008 WHO classification separates WM from LPL and uses the definition of WM adopted in the Second International Workshop on WM, that is, LPL with bone marrow involvement and an IgM monoclonal gammopathy of any concentration (2,7).

The WHO system also emphasizes that LPL/WM is essentially a diagnosis of exclusion, because many B-cell lymphomas can also mature into plasmacytoid or plasma cells containing immunoglobulin. Making the diagnosis

more complicated is the existence of nonsecretory LPL and LPL cases with monoclonal IgG and IgA gammopathy (2,8,9). Therefore, the class of serum monoclonal protein is not specified in the WHO definition.

Another unresolved problem for the diagnosis of LPL/WM is the quantity of the IgM paraprotein used to distinguish WM from other lymphomas and asymptomatic WM or monoclonal gammopathy of unknown significance (MGUS) (4,5). The cutoff suggested for WM ranges from 0.5 to 3 g/dL (3,4,10,11). However, because patients with a monoclonal spike of <0.5 g/dL may still be symptomatic, some authors suggested that a cutoff is not necessary as long as a monoclonal IgM protein is present (2,3,5).

Although LPL is frequently associated with monoclonal IgM gammopathy, the latter can be seen in many other conditions. In a study of 430 cases, Kyle and Garton (10) found that 56% were MGUS, 17% WM, 7% lymphoma, 5% chronic lymphocytic leukemia, 1% primary amyloidosis, and 14% lymphoproliferative disease. In their classification, the difference between WM and lymphoproliferative disease is based on the quantity of monoclonal IgM protein; the former has >3 g/dL of IgM, whereas the latter has <3 g/dL. In a study of 382 patients, Lin et al. (12) found

that 58.9% were LPL/WM, 20.2% were chronic lymphocytic leukemia/small lymphocytic lymphoma, and the remaining were other lymphomas.

Morphology

Whereas WM is regarded as a primary bone marrow disorder (Figs. 6.23.3 and 6.23.4) (2,5,13), LPL frequently presents as a node-based lymphoma (Fig. 6.23.5). There is no specific histologic pattern to distinguish LPL from other small-cell lymphomas, but LPL is characterized by a morphologic continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells (4). However, one cell type may be predominant in certain cases, so some authors suggest classifying LPL into lymphoplasmacytoid, lymphoplasmacytic, and polymorphous subtypes (13,14). Owen (5) considers this division as highly subjective and of doubtful prognostic significance. In rare cases, the plasma cell infiltrate is physically separate from the lymphoid aggregate (15). This pattern can easily mislead to erroneous diagnosis.

Because of the lack of pathognomonic features in LPL, the WHO system suggests that LPL should be diagnosed by excluding other lymphomas that may contain considerable numbers of lymphoplasmacytic cells.

Morphologically, small lymphocytic lymphoma/chronic lymphocytic leukemia is most similar to LPL. However, there are subtle differences between these tumors (3,5,16). First, LPL seldom shows proliferation centers. Second, tissue mast cells are usually increased in LPL (Fig. 6.23.6). Third, plasma cells and plasmacytoid cells are more numerous in LPL than in small lymphocytic lymphomas. Fourth, the plasma cells in LPL may contain periodic acid-Schiff (PAS)-positive intracytoplasmic and intranuclear inclusions (Russell and Dutcher bodies).

Other small-cell lymphomas that show plasma cell or plasmacytoid differentiation include nodal marginal

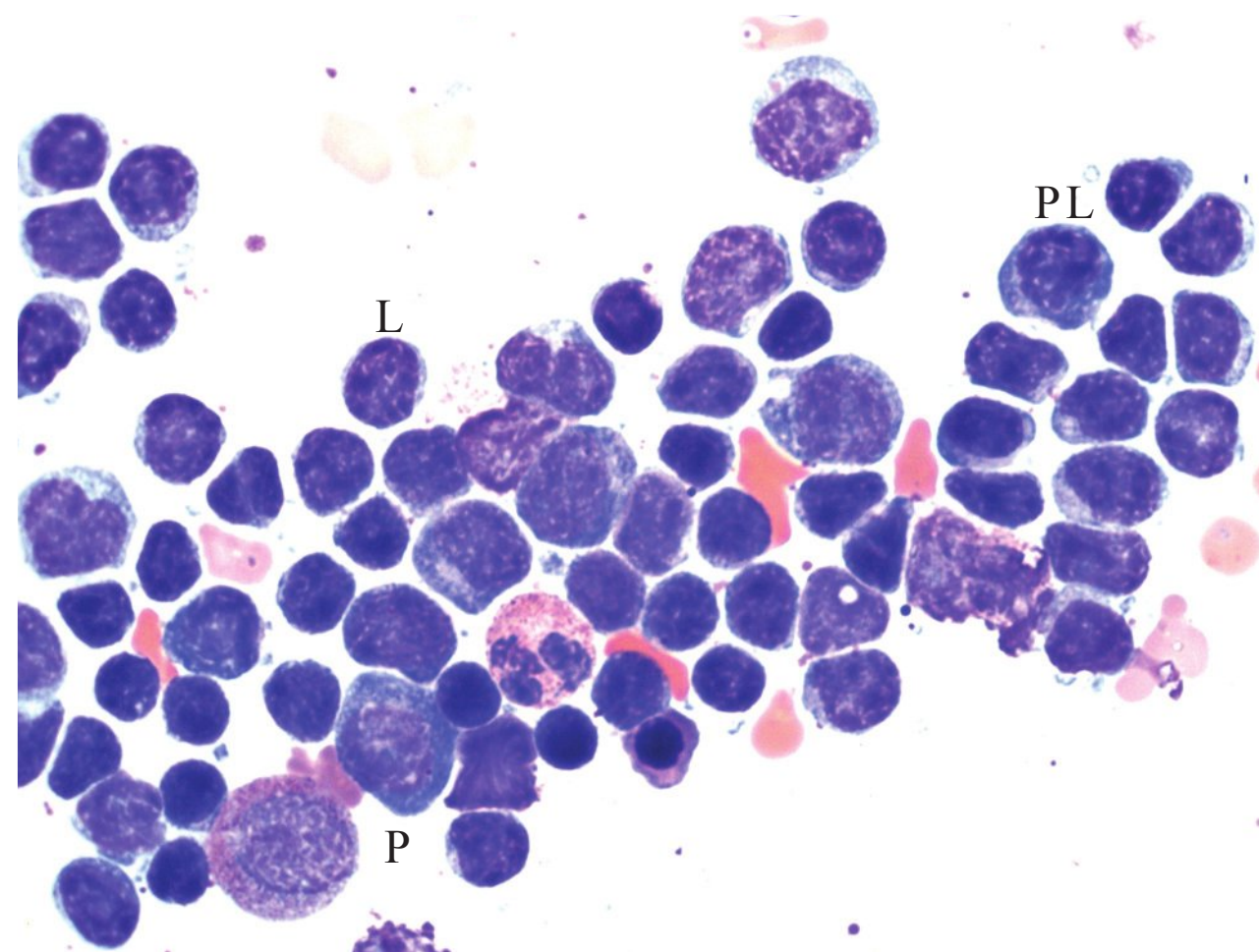


FIGURE 6.23.4 Bone marrow aspirate shows a spectrum of lymphocytes (L), plasmacytoid lymphocytes (PL), and plasma cells (P). Wright-Giemsa, 100× magnification.

zone B-cell lymphoma, extranodal marginal zone B-cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, and mantle cell lymphoma (1–5,12). The four criteria used to distinguish LPL from small lymphocytic lymphoma are also applicable in differentiating other lymphomas. In addition, the presence of follicular pattern, the marginal distribution of lymphoma cells, and monocytoid morphology should be looked for to exclude other lymphomas. Plasma cell myeloma should present as exclusively plasma cell infiltration in the bone marrow without lymphadenopathy. When the paraprotein produced is IgM, it should be designated IgM myeloma. In those cases, osteolytic bone lesion is frequently present (17).

Although small numbers of immunoblasts are frequently present in LPL, the marked increase in immunoblasts

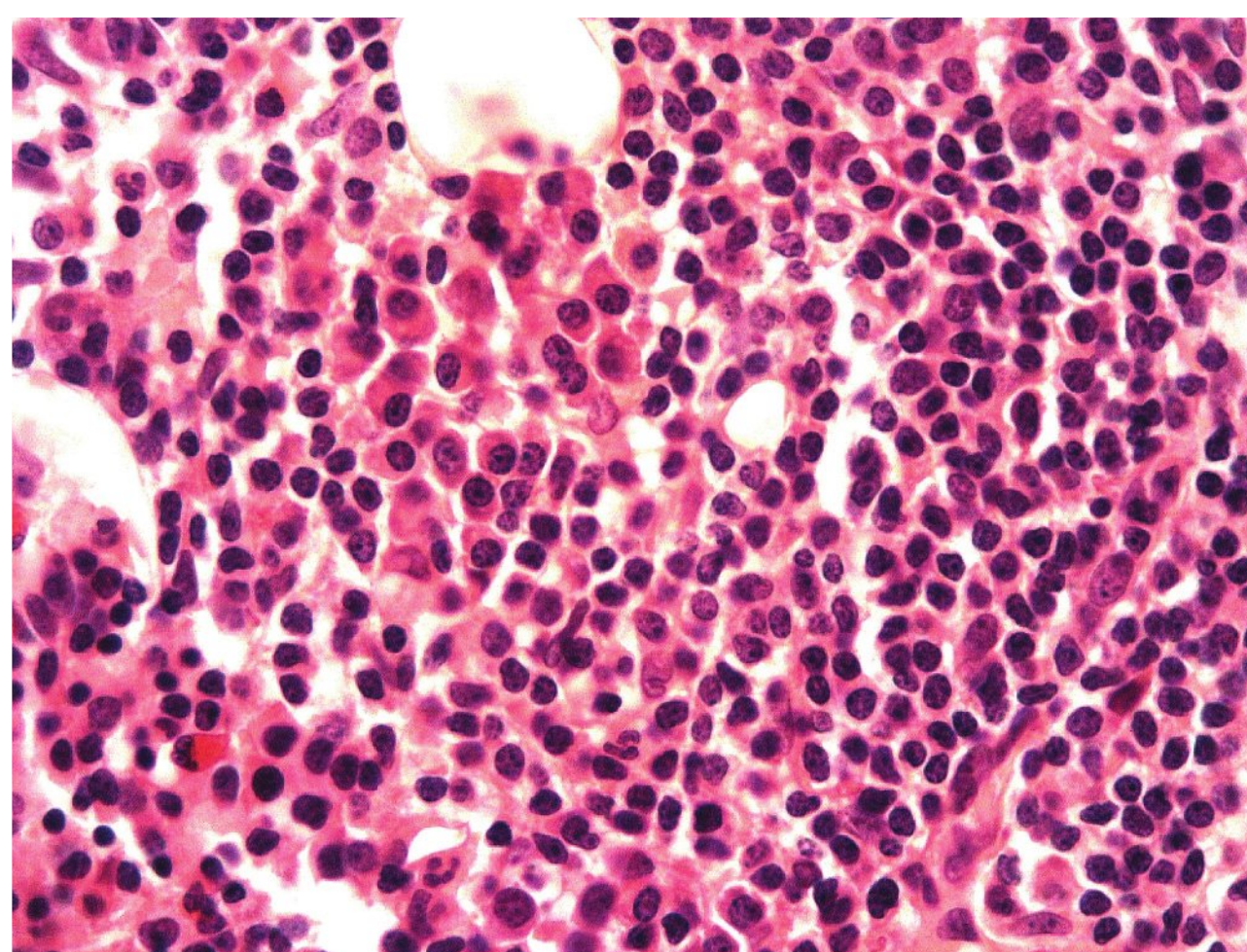


FIGURE 6.23.3 Bone marrow biopsy shows that normal hematopoiesis is replaced by interstitial lymphoplasmacytic infiltration. Note plasma cells are at the periphery of the lymphoid aggregate. Hematoxylin and eosin, 60× magnification.

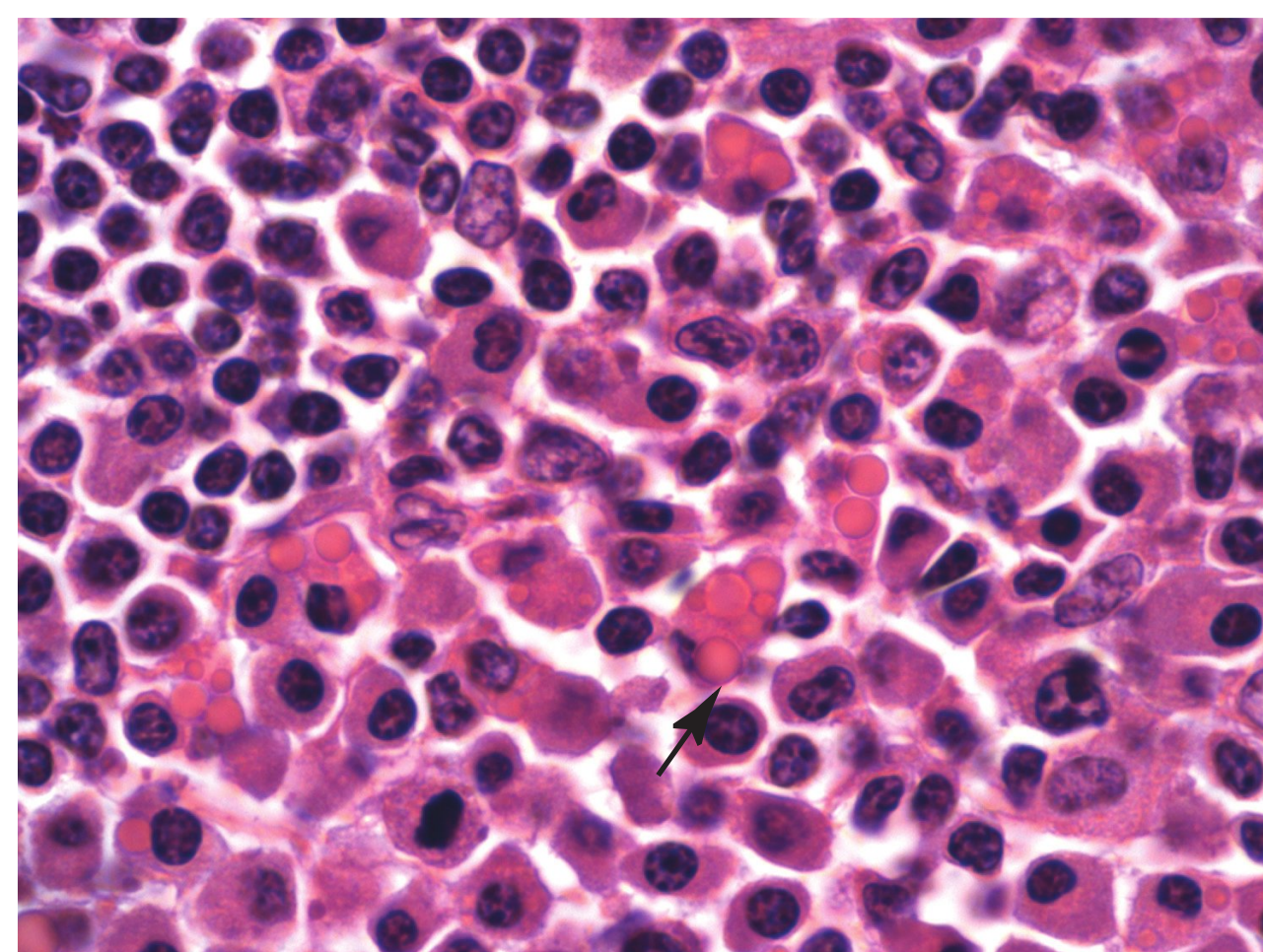


FIGURE 6.23.5 Lymph node biopsy shows a continuum of lymphocytes, plasmacytoid cells, and plasma cells. Some plasma cells contain numerous Russell bodies (white arrow). Hematoxylin and eosin, 100× magnification.

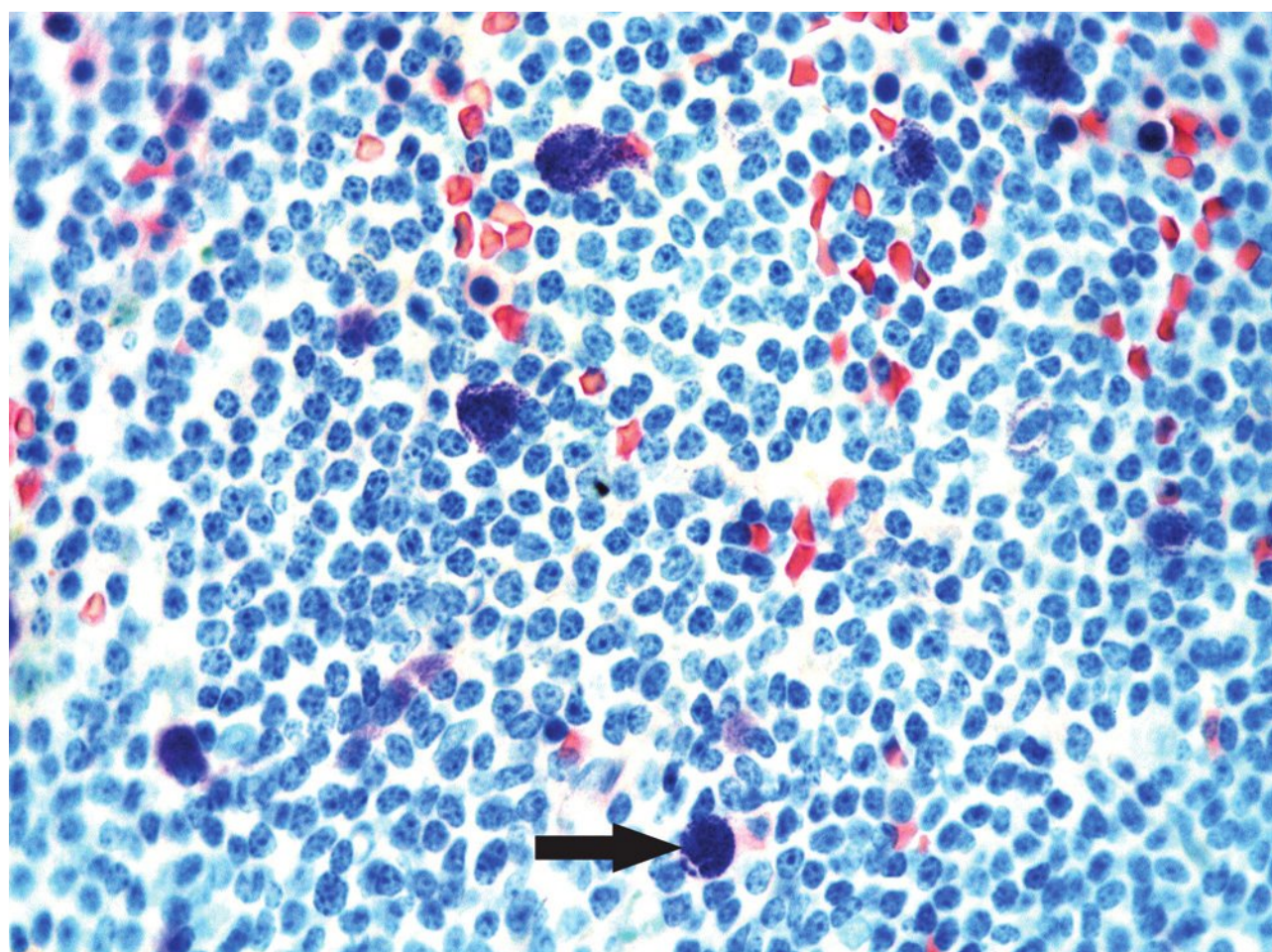


FIGURE 6.23.6 Bone marrow biopsy shows diffuse lymphoplasmacytic infiltration with many darkly stained mast cells (arrow). Some of the mast cells are out of focus. Giemsa, 60× magnification.

usually indicates a transformation to large cell (immunoblastic) lymphoma (Figs. 6.23.7 and 6.23.8) (1,18–21). On rare occasions, LPL may also transform into acute lymphoblastic leukemia (19,22), acute myelogenous leukemia (23–25), or chronic myelogenous leukemia (26).

By definition, WM is a bone marrow disorder, but only 65% of LPL patients have bone marrow involvement in one study (3). However, only 2% of WM patients show osteolytic bone lesions (27). The infiltration pattern in the bone marrow is usually nodular or interstitial, but paratrabecular and diffuse patterns can also be seen in a small number of cases (1,4,5). When a paratrabecular pattern is present in patients with lymphadenopathy, follicular lymphoma should be considered (5).

In the peripheral blood, lymphoplasmacytic cells may be present, particularly in WM and less frequently in LPL.

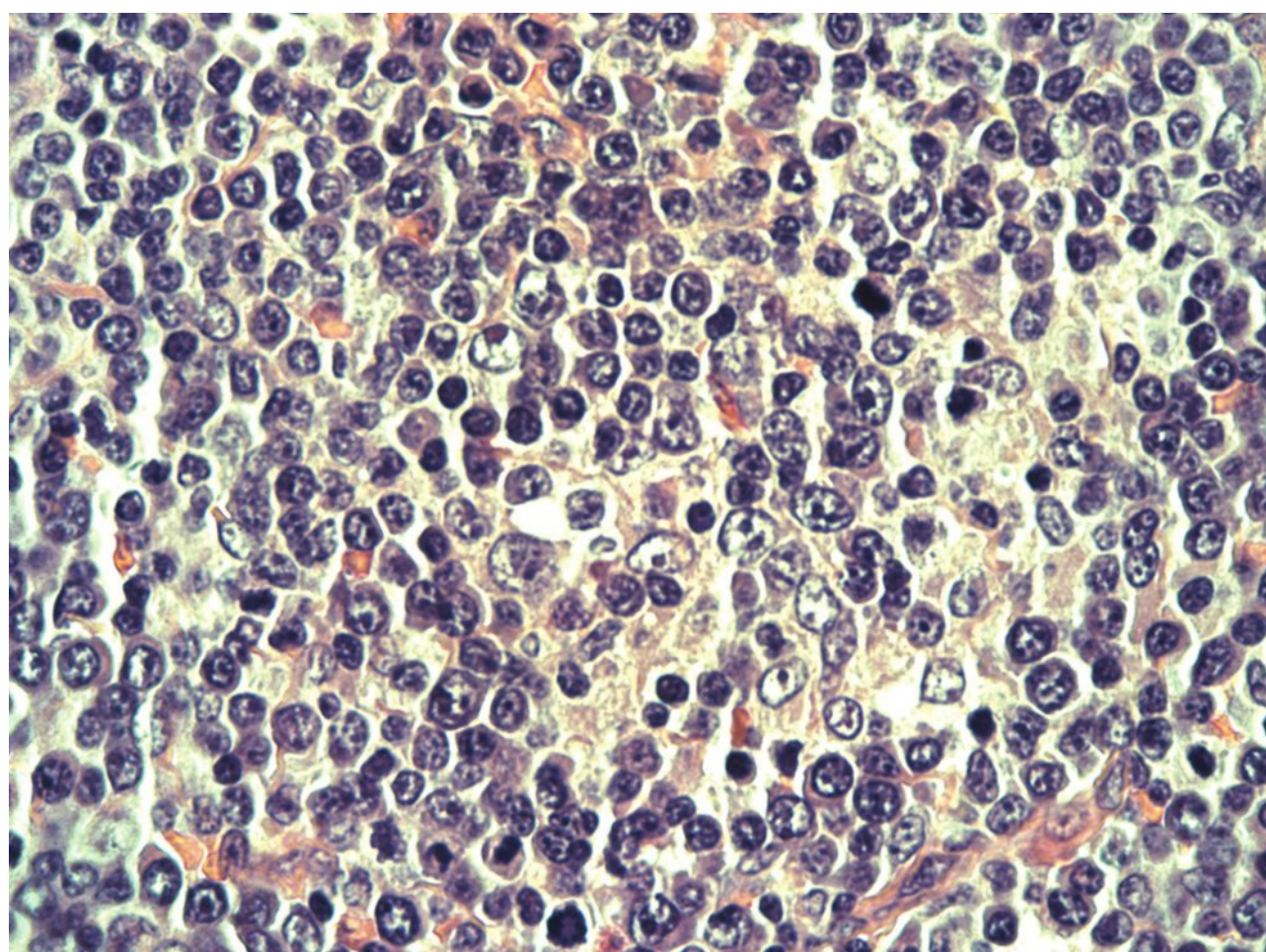


FIGURE 6.23.7 Lymph node biopsy shows immunoblastic transformation of LPL. Hematoxylin and eosin, 60× magnification.

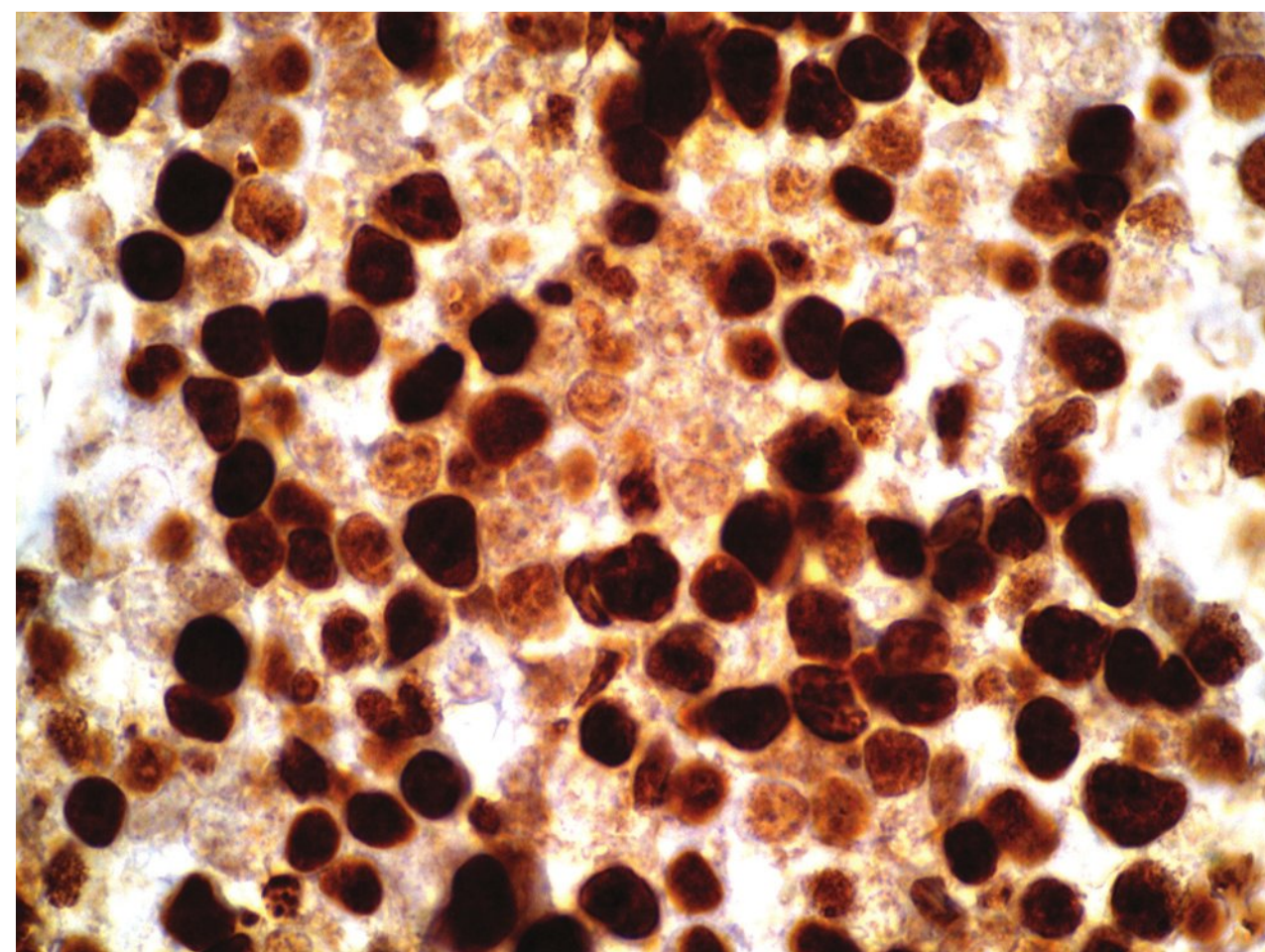


FIGURE 6.23.8 The immunoblasts are highlighted by Ki-67 stain in contrast to the lighter-stained small lymphocytes in the background. Immunoperoxidase, 100× magnification.

However, more striking features can be demonstrated in the erythrocytes, which may show rouleaux formation, depending on the quantity of the paraprotein. When the monoclonal IgM functions as a cold agglutinin, hemagglutination may occur (28).

In the current case, both the lymph node biopsy and the bone marrow biopsy showed a continuum of small lymphocytes, plasmacytoid lymphocytes, and plasma cells. Some of the plasma cells revealed bunches of Russell bodies. Immunohistochemical stains demonstrated a B-cell population with positive CD20 staining and a plasma cell population that presented with a monoclonal k immunophenotype. However, the dual surface and cytoplasmic monoclonal immunoglobulin pattern, as demonstrated by flow cytometry, is most helpful in diagnosing LPL/WM in this case.

According to the laboratory data, the patient had anemia, elevation of reticulocyte count, serum bilirubin, and lactate dehydrogenase. These findings are consistent with hemolytic anemia. This phenomenon can be explained by the presence of high-titered cold agglutinin, which, in turn, is probably associated with the monoclonal IgM. It is interesting to see that the low quantity of IgM, such as in this case, may cause a disproportionately high titer of cold agglutinin.

Immunophenotype

LPL/WM is characterized by the coexistence of two immunophenotypes: mature lymphocytes and plasma cells (11). In immunohistochemical stains, the lymphocytes show B-cell markers (CD20) (Fig. 6.23.9) and the plasma cells demonstrate a monoclonal cytoplasmic immunoglobulin pattern (Fig. 6.23.10). By flow cytometry, the immunophenotype represents the combination of these two cell types; namely, the coexistence of both B-cell and plasma cell markers. Because of the presence of a spectrum of lymphocytes, plasmacytoid lymphocytes, and plasma cells, both surface

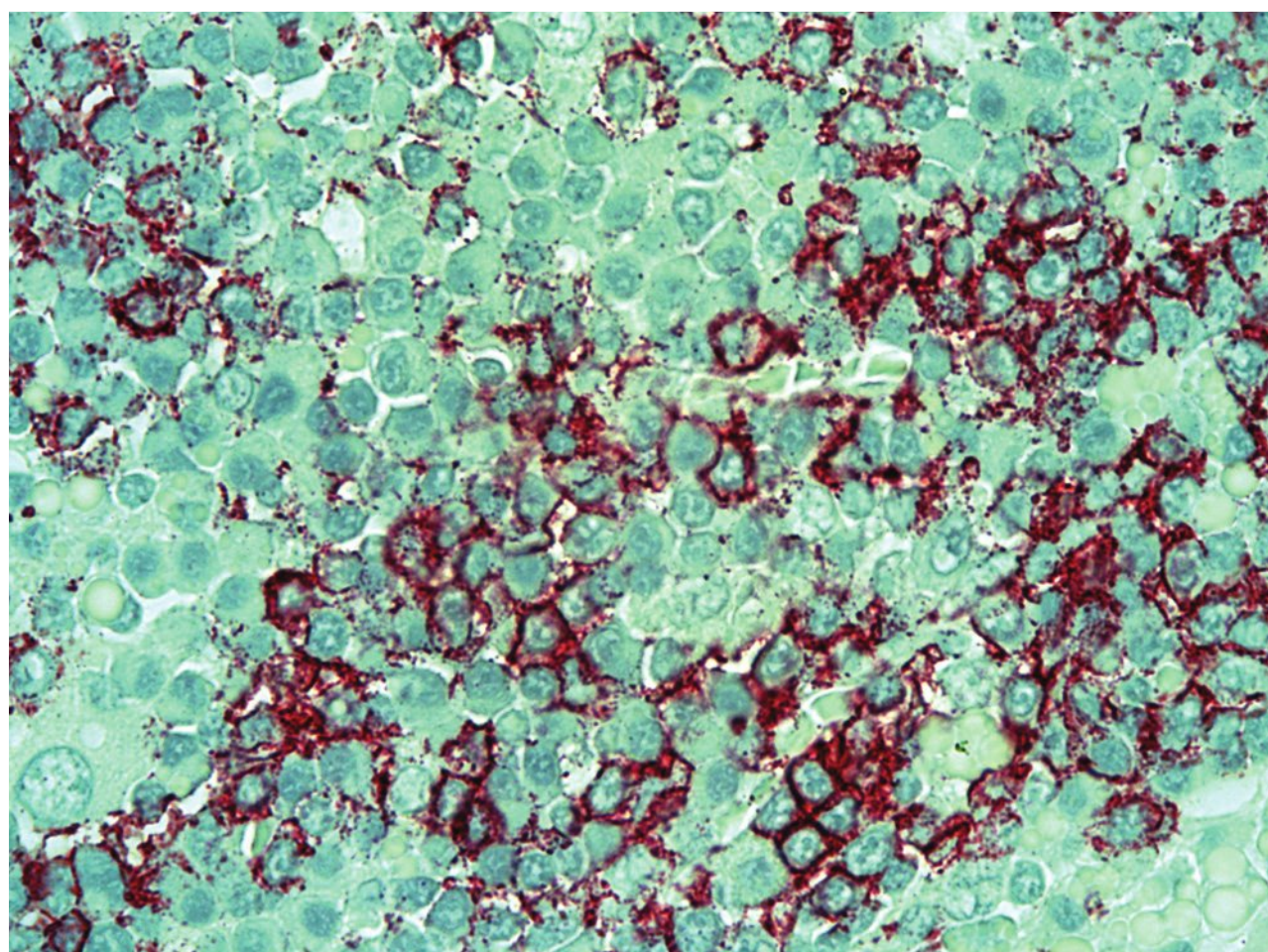


FIGURE 6.23.9 Lymph node biopsy shows CD20 staining on small lymphocytes but not plasma cells. Immunoalkaline phosphatase, 60× magnification.

and cytoplasmic monoclonal immunoglobulin patterns are present. More specifically, the percentages of cells with monoclonal surface immunoglobulin and monoclonal cytoplasmic immunoglobulin overlap (for instance, 70% of the former and 50% of the latter). This pattern may be explained by the existence of some lymphoplasmacytic cells that express both surface and cytoplasmic immunoglobulin. San Miguel et al. (11) suggest that the coexistence of clonal B lymphocytes and clonal plasma cells is specific for WM and has not been reported in any other lymphoproliferative disorders, including LPL. However, others have found that this phenomenon is also commonly seen in LPL cases (1). In fact, marginal zone lymphomas also show the same pattern (29).

The positive B-cell markers demonstrated in the lymphoid cells include CD19, CD20, CD22, CD24, and HLA-DR

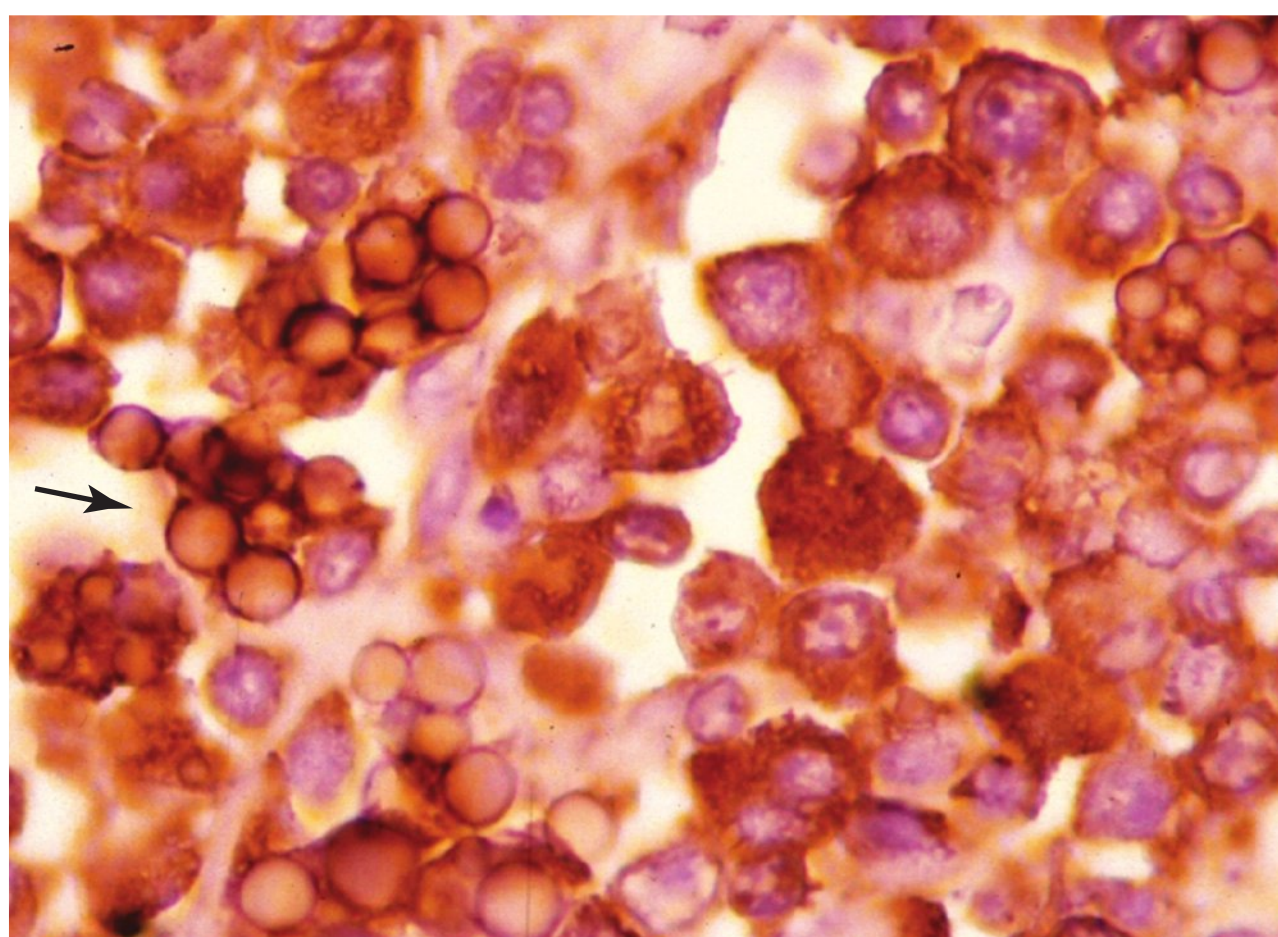


FIGURE 6.23.10 Lymph node biopsy shows κ light-chain staining of the plasma cells, but not the small lymphocytes. Many Russell bodies (arrow) are highlighted by the staining. λ light-chain stain is negative (data not shown). Immunoperoxidase, 100× magnification.

(3,4,10,12,16,18,30). CD27, CD79a, PAX-5, and bcl-2 have also been demonstrated by immunohistochemistry (4,5). The presence of a memory B-cell marker (CD27) is consistent with the hypothesis that LPL/WM is derived from postgerminal center B cells (5). A high percentage of cases also express FMC-7 and CD25. The presence of the above markers may raise the possible diagnosis of hairy cell leukemia, but other markers for this disease, such as CD103 and CD11c, are usually negative.

Other negative markers that are useful in the differential diagnosis include CD5, CD10, CD23, and cyclin D1, which may help to exclude small lymphocytic lymphoma/chronic lymphocytic leukemia, mantle cell lymphoma, and follicular lymphoma (4,11). However, San Miguel et al. (11) found that symptomatic WM cases frequently showed up-regulation of CD5 and down-regulation of both FMC-7 and CD25. Konoplev et al. (31) reported that CD11c and CD23 were present in 81% and 61% of 75 cases of LPL/WM, respectively. Morice et al. (15) identified CD5 in 43% and CD23 in 52% of 35 LPL cases, though most cases showed only partial expression.

Besides the demonstration of a monoclonal cytoplasmic pattern, plasma cells usually show an immunophenotype of CD19+, CD38+, CD138+, CD45+, CD56- (7,11,15). In a subset of plasma cells, CD20 is also positive. This immunophenotype differs from that of the plasma cells in plasma cell myeloma, which usually shows dim or negative CD45, negative CD19, and frequently positive CD56.

Mast cells are frequently increased in cases of LPL/WM, but they usually reveal a normal immunophenotype (CD2-, CD25-, CD35-, CD63+ dim, and CD65+ dim); thus, mast cells are not part of the malignant clone (11). However, other studies found that mast cells may induce WM cell proliferation and/or tumor colony formation through, in part, constitutive expression of CD40 ligand (13).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry can demonstrate the double surface and cytoplasmic immunoglobulin pattern and is very helpful in diagnosing LPL/MW. However, immunohistochemistry can demonstrate two cell populations: The lymphoid cells express CD20, while the plasma cells show a monoclonal immunoglobulin pattern. Therefore, both techniques are helpful in achieving a correct diagnosis, but the results obtained from flow cytometry are sometimes more definitive than those from immunohistochemistry.

Molecular Genetics

Whereas LPL is closely related to WM, the nonsecretory LPL is a biologically distinct entity. A translocation t(9;14) (p13;q32) has been reported in up to 50% of those cases (32,33). However, this abnormality can also be detected in other lymphomas with plasmacytoid differentiation (32,34). This translocation is characterized molecularly as the translocation of the PAX-5 gene in chromosome 9 to the IgH gene in chromosome 14. PAX-5 encodes the B-cell-specific activator protein that is important in early B-cell development. The PAX-5 product abrogates the production of the J peptide (34) and down-regulates IgH



transcription (35,36). Therefore, no monoclonal IgM gammopathy is present in LBL cases with this translocation. In contrast, t(9;14) and IgH translocation have not been found by karyotyping and fluorescence hybridization technique in pure WM cases (3,5,6).

Until recently, the only recurrent genetic aberration in WM cases is deletion of the long arm of chromosome 6 (6). Up to 50% of WM cases have this deletion, and most cases have deletion at 6q21 (6,37). However, a recent study of 11 LPL samples with genome-wide microarray-based comparative genomic hybridization analysis demonstrated deletion of chromosome 17 (2 cases), gain of 3q (2 cases), and gain of chromosome 7, in addition to deletion of 6q (2 cases) (38).

LBL/WM have immunoglobulin heavy- and light-chain gene rearrangement with somatic hypermutation in the variable regions (6,9,39), which indicates that the tumor cells are of the postgerminal center origin. However, the switch mgene is not rearranged. The lack of physiologic isotype switching explains why only monoclonal IgM protein is produced in WM (6). In DNA ploidy studies, aneuploidy is seldom found in WM cases except for the aggressive variants of WM (6).

Although plasma cell myeloma seems to be close to WM in terms of production of monoclonal gammopathy and of bone marrow involvement by neoplastic plasma cells, they are very different genotypically. For instance, myeloma may show IgH translocation and switch mchain gene rearrangement, and is frequently aneuploid (5,6). Even though B cells and plasma cells are considered the integral part of LPL/WM, they express different patterns in gene expression profiling; the B cells cluster with chronic lymphocytic leukemia, while the plasma cells cluster with multiple myeloma (40)

The salient features for laboratory diagnosis of LPL are summarized in Table 6.23.1.

Clinical Manifestations

LBL/WM is usually seen in elderly patients with a median age of 63 years (range 25 to 92), and there is a slight predominance of male patients (41). The incidence rate is higher among Caucasians, and only 5% of all patients are of African descent (13). Recent evidence suggests that LBL/WM may be a familial disease showing multigenerational clustering of WM and other B-cell–lymphoproliferative diseases. In a study of 257 patients, approximately 18.7% had a first-degree relative with such diseases (42). A Swedish study of 2144 LPL/WM patients found that the first degree relatives of these patients had 20-fold, 3.0-fold, 3.4-fold and 5.0-fold increased risks of developing LPL/WM, non-Hodgkin lymphomas, chronic lymphocytic leukemia, and monoclonal gammopathy with undetermined significance, respectively (43). The same study group found that autoimmune diseases, such as systemic sclerosis, Sjögren syndrome, autoimmune hemolytic anemia, polymyalgia rheumatica and giant cell arteritis, were also associated with an increased risk of LPL/WM (44).

WM differs from plasma cell myeloma in its slowly progressive clinical course; the patient may not require any treatment for several years or may require only

TABLE 6.23.1

Salient Features for Laboratory Diagnosis of LPL

- 1. A small-cell lymphoma with a wide spectrum of lymphoplasmacytic infiltration, including small lymphocytes, plasmacytoid lymphocytes, and plasma cells.
- 2. No special patterns, such as follicular, marginal zone, and proliferation center, diagnostic for other lymphomas are present.
- 3. Bone marrow may show interstitial, nodular, diffuse, and occasional paratrabecular infiltration pattern by lymphoplasmacytic cells.
- 4. Mast cells are frequently increased in bone marrow.
- 5. The plasma cells may contain prominent Russell bodies or Dutcher bodies.
- 6. Immunohistochemical stains demonstrate two populations: CD20-positive small lymphocytes and monoclonal k- or l-positive plasma cells.
- 7. Flow cytometry shows a dual monoclonal surface and cytoplasmic immunoglobulin pattern.
- 8. Characteristic immunophenotype of the B cells includes positive reactions to CD19, CD20, CD22, CD24, CD38, CD79a, PAX5, and HLA-DR and negative reactions to CD5, CD10, CD23, and cyclin D1. The immunophenotype of plasma cells includes CD19+, CD38+, CD138+, CD45+, CD56- .
- 9. A monoclonal IgM gammopathy is supportive of the diagnosis of LPL, but many cases of LPL are nonsecretory for monoclonal immunoglobulin.
- 10. Fifty percent of the nonsecretory LPL show a karyotype of t(9;14)(p13;q32).
- 11. The only recurrent genetic aberration in WM cases is deletion of the long arm of chromosome 6 (–6q21).

LPL, lymphoplasmacytic lymphoma; WM, Waldenström macroglobulinemia.

plasmapheresis to reduce the concentration of macroglobulin. The initial clinical symptoms are usually vague and nonspecific, such as weakness, anorexia, and weight loss. However, in the later stage, there are specific clinical manifestations that are related to the paraprotein and subsequent hematologic changes.

The best known symptom in WM is the hyperviscosity syndrome. However, in most patients this syndrome only appears when the serum viscosity reaches 4 units (four times as viscous as water) or when IgM concentration exceeds 3 g/dL (27,30). In contrast, patients with a high concentration of IgA or IgG (≥5 g/dL) may also have this syndrome. In addition, the molecular configuration also contributes to high viscosity; for instance, hyperviscosity is frequently associated with the polymers of IgA and with molecular asymmetry of IgG (17). The symptoms of this syndrome include bleeding diathesis, retinopathy, hypervolemia, congestive heart failure, and various neurologic

symptoms that may progress from headache and confusion to coma. The blurred vision seen in WM patients is thought to reflect rouleaux formation or “sludging” in ocular vessels (45).

The bleeding tendency in WM is multifactorial. Evidence suggests that coating the platelets by monoclonal IgM protein may cause platelet aggregation defect (46), binding of IgM molecules to fibrin monomers may inhibit fibrin polymerization (46), and immunoadsorption of factor VIII by neoplastic lymphocytes also interferes with the normal coagulation process (47).

Some monoclonal macroglobulins may function as antibodies against red cell antigens I and i (cold hemagglutinins), thus causing hemagglutination (Fig. 6.23.11) in the cold and leading to hemolysis (28). In cases showing these autoantibodies without hepatosplenomegaly, lymphadenopathy, and bone marrow infiltration, the condition is designated as the cold agglutinin syndrome. In 15% of patients, the monoclonal macroglobulin protein may precipitate in the cold (Fig. 6.23.12); this type of protein is called type I cryoglobulin (30,48). Patients with cryoglobulinemia may show the Raynaud phenomenon, cold hypersensitivity, cold urticaria, cold purpura, or glomerulonephritis in <5% of patients (27,30).

Renal insufficiency can be seen in patients with WM, but it is less frequent than in patients with plasma cell myeloma, because Bence–Jones proteinuria is usually <2 g/24 hours or 1.0 g/dL, and hypercalcemia is less frequently seen in WM cases (30,49). In myeloma, tubular degeneration and cast formation are the major changes in the kidney, whereas glomerular lesions due to IgM deposition are the more constant finding in WM (49). Other extramedullary sites involved in WM include spleen, skin, lung, tonsil, colon (Fig. 6.23.13), liver, gallbladder, and soft tissues (13).

Approximately 10% of patients may develop polyneuropathy (30). The pathogenesis of neuropathy may be due to plasma cell infiltration, antibodies against various

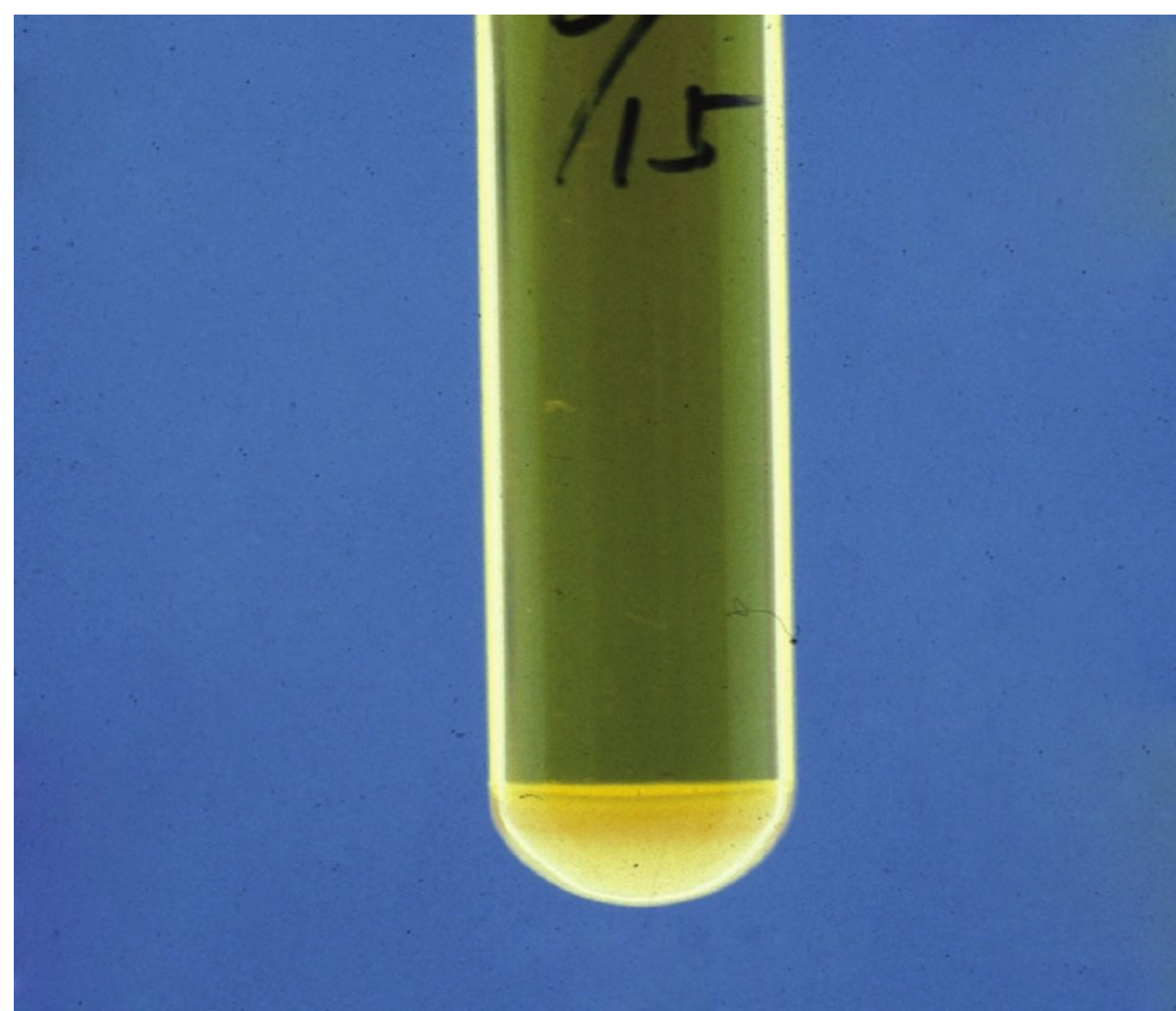


FIGURE 6.23.12 Test tube of serum stored at 4°C overnight showing cryoprecipitate at the bottom.

glycoproteins or glycolipids, or the peripheral nerves and amyloid deposition. Amyloid light chain amyloidosis should be suspected in all LPL patients with nephrotic syndrome, cardiomyopathy, hepatomegaly, or peripheral neuropathy (13).

In cases of LPL without macroglobulinemia, the above-mentioned clinical symptoms may not be manifested, and the clinical course is similar to other low-grade lymphoproliferative disorders.

As in plasma cell myeloma, Kaposi sarcoma–associated herpesvirus has been identified in bone marrow biopsies from patients with WM, and a viral interleukin-6 produced by Kaposi sarcoma–associated herpesvirus may participate in the proliferation of malignant cells in WM (18,30). When LPL is associated with type II mixed cryoglobulinemia, it

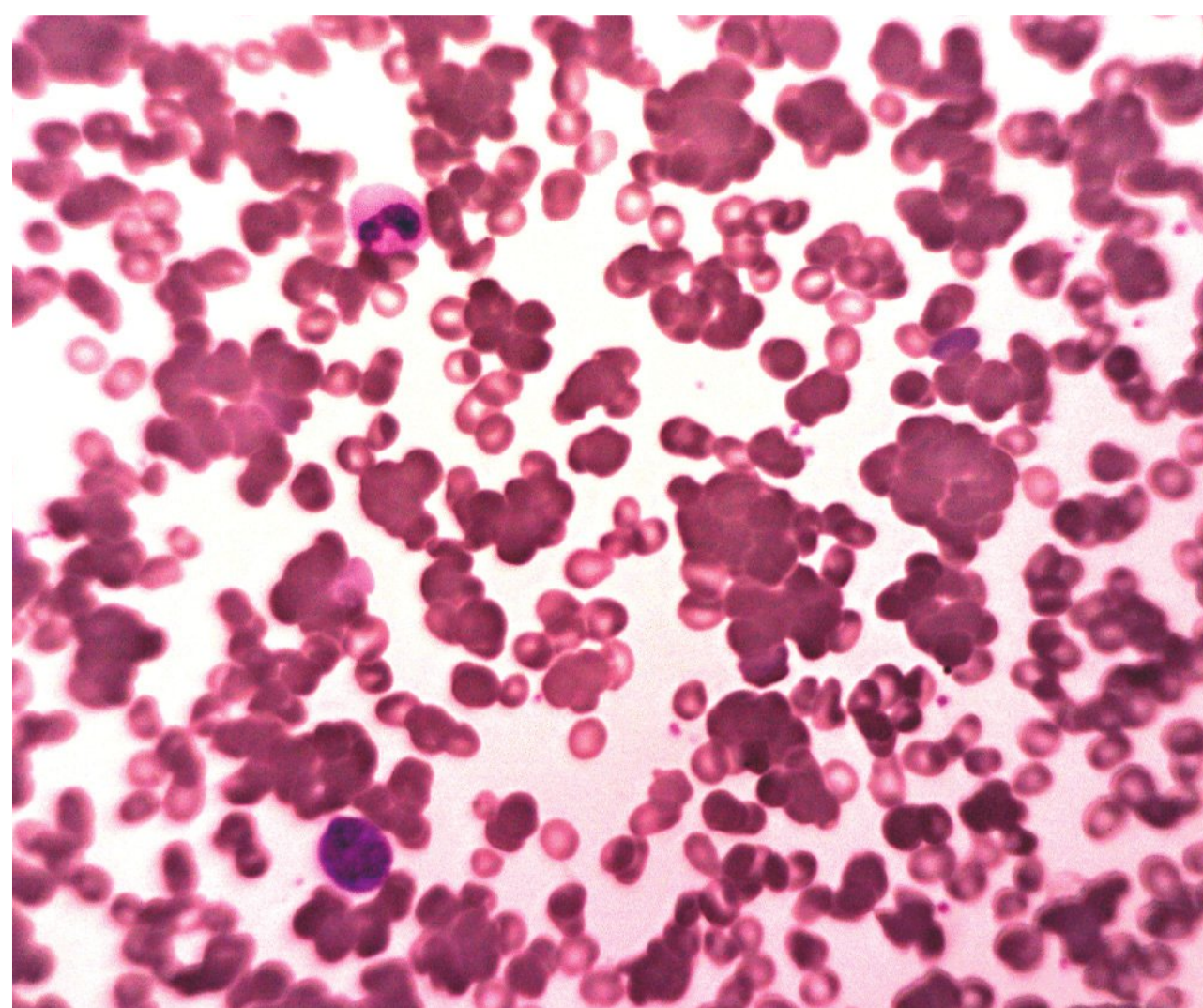


FIGURE 6.23.11 Peripheral blood smear shows hemagglutination. Wright–Giemsa, 40× magnification.

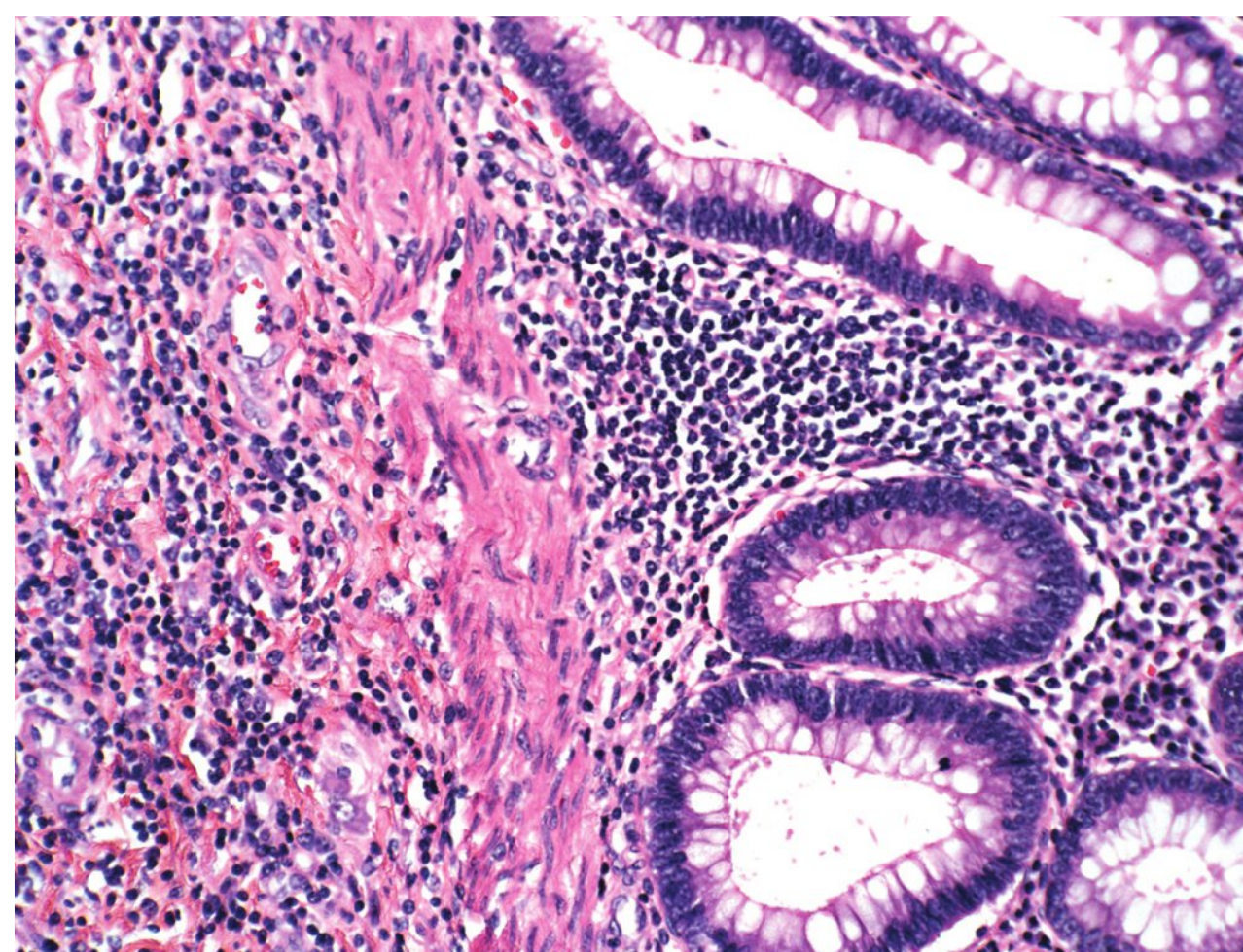


FIGURE 6.23.13 Colon biopsy shows mucosal and submucosal infiltration by LPL cells. Hematoxylin and eosin, 20× magnification.

may be associated with hepatitis C virus infection; treatment of patients with interferon to reduce viral load has shown to induce regression of the lymphoma (50).

REFERENCES

- Berger F, Isaacson PG, Piris MA, et al. Lymphoplasmacytic lymphoma/Waldenström macroglobulinemia. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:132–134.
- Owen RG, Treon SP, Al-Katib A, et al. Clinicopathologic definition of Waldenström macroglobulinemia: consensus panel recommendations from the second international workshop on Waldenström's macroglobulinemia. *Semin Oncol*. 2003;30:110–115.
- Pangalis GA, Kyrtsos MC, Kontopidou FN, et al. Differential diagnosis of Waldenström's macroglobulinemia from other low-grade B-cell lymphoproliferative disorders. *Semin Oncol*. 2003;30:201–205.
- Remstein ED, Hanson CA, Kyle RA, et al. Despite apparent morphologic and immunophenotypic heterogeneity, Waldenström's macroglobulinemia is consistently composed of cells along a morphologic continuum of small lymphocytes, plasmacytoid lymphocytes and plasma cells. *Semin Oncol*. 2003;30:182–186.
- Owen RG. Developing diagnostic criteria in Waldenström's macroglobulinemia. *Semin Oncol*. 2003;30:196–200.
- Schop RFJ, Fonseca R. Genetics and cytogenetics of Waldenström's macroglobulinemia. *Semin Oncol*. 2003;30:142–145.
- Serdlow SH, Berger F, Pileri SA, et al. Lymphoplasmacytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:194–195.
- Papamichael D, Norton AJ, Foran JM, et al. Immunocytoma: a retrospective analysis from St. Bartholomew's Hospital—1972 to 1996. *J Clin Oncol*. 1999;17:2847–2853.
- Sahota S, Garand R, Bataille R, et al. V_H gene analysis of clonally related IgM and IgG from human lymphoplasmacytoid B-cell tumors with chronic lymphocytic leukemia features and high serum monoclonal IgG. *Blood*. 1998;91:236–243.
- Kyle RA, Garton JP. The spectrum of IgM monoclonal gammopathy in 430 cases. *Mayo Clin Proc*. 1987;62:719–731.
- San Miguel JF, Vidriales MB, Ocio E, et al. Immunophenotypic analysis of Waldenström's macroglobulinemia. *Semin Oncol*. 2003;30:187–195.
- Lin P, Hao S, Handy BC, et al. Lymphoid neoplasms associated with IgM paraprotein: a study of 382 patients. *Am J Clin Pathol*. 2005;123:200–205.
- Gertz MA, Merlini G, Treon SP. Amyloidosis and Waldenström's macroglobulinemia. *Hematology*. 2004. Washington, DC: American Society of Hematology; 2004:257–282.
- Lin P, Bueso-Ramos C, Wilson C, et al. Waldenström macroglobulinemia involving extramedullary sites: morphologic and immunophenotypic findings in 44 patients. *Am J Surg Pathol*. 2003;27:1104–1113.
- Morice WG, Chen D, Kurtin PJ, et al. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correlation with Waldenström's macroglobulinemia. *Mod Pathol*. 2009;22:807–816.
- Pangalis GA, Angelopoulou MK, Vassilakopoulos TP, et al. B-chronic lymphocytic leukemia, small lymphocytic lymphoma, and lymphoplasmacytic lymphoma, including Waldenström macroglobulinemia: a clinical, morphologic and biologic spectrum of similar disorders. *Semin Hematol*. 1999;36:104–114.
- Fudenberg HH, Virella G. Multiple myeloma and Waldenström macroglobulinemia: unusual presentations. *Semin Hematol*. 1980;17:63–79.
- Dimopoulos MA, Galani E, Matsouka C. Waldenström macroglobulinemia. *Hematol Oncol Clin North Am*. 1999;13:1351–1366.
- Leonhard SA, Muhleman AF, Hurtibise PE, et al. Emergence of immunoblastic sarcoma in Waldenström's macroglobulinemia. *Cancer*. 1986;45:3102–3107.
- Abe M, Takahashi K, Mori N, et al. "Waldenström macroglobulinemia" terminating in immunoblastic sarcoma. A case report. *Cancer*. 1982;49:2580–2586.
- Emmerich B, Pems M, Wust I, et al. Conversion of an IgM secreting immunocytoma to a high grade malignant lymphoma of immunoblastic type. *Blut*. 1983;46:81–84.
- Madan RA, Chang VT, Yook C, et al. Waldenström's macroglobulinemia evolving into acute lymphoblastic leukemia: a case report and a review of the literature. *Leukemia*. 2004;18:1433–1435.
- Salberg D, Kurtides ES, McKeever WP. Monomyelocytic leukemia in an unrelated case of Waldenström's macroglobulinemia. *Arch Intern Med*. 1997;137:514–516.
- Horsman DE, Card RT, Skinnider LF. Waldenström's macroglobulinemia terminating in acute leukemia. A report of 3 cases. *Am J Hematol*. 1983;15:97–101.
- Majumdar G, Slater NG. Waldenström's macroglobulinemia terminating in acute myeloid leukaemia: report of a case and review of the literature. *Leuk Lymphoma*. 1993;9:513–516.
- Vitali C, Bombardieri S, Spremolla G. Chronic myeloid leukemia in Waldenström's macroglobulinemia. *Arch Intern Med*. 1981;141:1349–1351.
- Dimopoulos MA, Alexanian R. Waldenström's macroglobulinemia. *Blood*. 1994;83:1452–1459.
- Pruzanski W, Katz A. Cold agglutinins: antibodies with biological diversity. *Clin Immunol Rev*. 1984;3:131–168.
- Berger F, Traverse-Glehen A, Felman P, et al. Clinicopathologic features of Waldenström's macroglobulinemia and marginal zone lymphoma: Are they distinct or the same entity? *Clin Lymphoma*. 2005;5:220–224.
- Dimopoulos MA, Panayiotidis P, Mouloupoulos LA, et al. Waldenström's macroglobulinemia: clinical features, complications, and management. *J Clin Oncol*. 2000;18:214–226.
- Konoplev S, Medeiros LJ, Bueso-Ramos CE, et al. Immunophenotypic profile of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia. *Am J Clin Pathol*. 2005;124:414–420.
- Offit K, Parsa NZ, Filippa D, et al. t(9;14)(p13;q32) denotes a subset of low-grade non-Hodgkin's lymphoma with plasmacytoid differentiation. *Blood*. 1992;80:2594–2599.
- Lida S, Rao PH, Ueda R, et al. Chromosomal rearrangement of the PAX-5 locus in lymphoplasmacytic lymphoma with t(9;14)(p13;q32). *Leuk Lymphoma*. 1999;34:25–33.
- Morrison AM, Jager U, Chott A, et al. Deregulated PAX-5 transcription from a translocated IgH promoter in marginal zone lymphoma. *Blood*. 1998;92:3865–3878.
- Max EE, Wakatsuki Y, Neurath MF, et al. The role of BSAP in immunoglobulin isotype switching and B-cell proliferation. *Curr Top Microbiol Immunol*. 1995;194:449–458.

36. Neurath MF, Stuber ER, Strober W. BSAP: a key regulator of B-cell development and differentiation. *Immunol Today*. 1995;16:564–569.
37. Wong KF, So CC. Waldenstrom macroglobulinemia with karyotypic aberrations involving both homologous 6q. *Cancer Genet Cytogenet*. 2001;124:137–139.
38. Buckley PG, Walsh SH, Laurell A, et al. Genome-wide microarray-based comparative genomic hybridization analysis of lymphoplasmacytic lymphoma reveals heterogeneous aberrations. *Leuk Lymphoma*. 2009;50:1528–1534.
39. Sahota SS, Forconi F, Ottensmeier CH, et al. Typical Waldenstrom macroglobulinemia is derived from a B-cell arrested after cessation of somatic mutation but prior to isotype switch events. *Blood*. 2002;100:1505–1507.
40. Gutierrez NC, Ocio EM, de Las RJ, et al. Gene expression profiling of B lymphocytes and plasma cells from Waldenström's macroglobulinemia: comparison with expression patterns of the same cell counterparts from chronic lymphocytic leukemia, multiple myeloma and normal individuals. *Leukemia*. 2007;21:541–549.
41. Merlini G, Baldini L, Broglia C, et al. Prognostic factors in symptomatic Waldenstrom's macroglobulinemia. *Semin Oncol*. 2003;30:211–215.
42. Treon SP, Hunter ZR, Aggarwal A, et al. Characterization of familial Waldenstrom's macroglobulinemia. *Ann Oncol*. 2006;17:488–494.
43. Kristinsson SY, Björkholm M, Goidin LR, et al. Risk of lymphoproliferative disorders among firsts-degree relatives of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia patients: a population-based study in Sweden. *Blood*. 2008;112:3052–3056.
44. Kristinsson SY, Koshiol J, Björkholm M, et al. Immune-related and inflammatory conditions and risk of lymphoplasmacytic lymphoma or Waldenström macroglobulinemia. *J Natl Cancer Inst*. 2010;102:557–567.
45. Grogan TM, Spier CM. B-cell immunoproliferative disorders, including multiple myeloma and amyloidosis. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1557–1587.
46. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 3–1990. A 66-year-old woman with Waldenstrom's macroglobulinemia, diarrhea, anemia, and persistent gastrointestinal bleeding. *N Engl J Med*. 1990;322:183–192.
47. Brody JI, Haidar ME, Rossman RE. A hemorrhagic syndrome in Waldenstrom's macroglobulinemia secondary to immunoabsorption of factor VIII: recovery after splenectomy. *N Engl J Med*. 1979;300:408–410.
48. Malchesky PS, Clough JD. Cryoimmunoglobulins: properties, prevalence in disease and removal. *Cleve Clin Q*. 1985;52:175–192.
49. Bergsagel DE. Macroglobulinemia. In: Williams WJ, Butler E, Erslev AH, et al., eds. *Hematology*. 4th ed. New York: McGraw-Hill; 1990:1141–1145.
50. Mazzaro C, Franzin F, Tulissi P, et al. Regression of monoclonal B-cell expansion in patients affected by mixed cryoglobulinemia responsive to alpha-interferon therapy. *Cancer*. 1996;77:2604–2613.

CASE 24

Plasma Cell Myeloma and Plasmacytoma

CASE HISTORY

An 81-year-old man presented with back pain as well as right arm and shoulder pain. He was admitted to the hospital for further examination 3 years ago. During the first admission, a diagnosis of multiple myeloma (MM) was made by bone marrow biopsy. Bone survey showed lytic lesions in the right proximal femur, right humerus, and skull. Degenerative disk disease was found in the cervical spine.

Laboratory finding on admission included decreased immunoglobulin (Ig) levels in IgG (368 mg/dL), IgA (20 mg/dL), and IgM (9 mg/dL). Light-chain Igs were not quantified. However, immunofixation demonstrated monoclonal gammopathy, and urine electrophoresis revealed Bence-Jones protein. Serum calcium at that time was 9.8 mg/dL.

Subsequently, the patient underwent multiple therapeutic regimens, including palliative radiation therapy to the right arm and treatment with prednisone, melphalan, thalidomide, and bortezomib. After extensive treatment, the patient had a complete remission for approximately 1 year with <5% normal-looking plasma cells in the bone marrow on two occasions.

One year later, the patient suffered from compression fracture of the L2 vertebral body, and follow-up study found that he had elevated b_2 microglobulin (B_2M ; 10.8 mg/L) and lactate dehydrogenase (LDH; 837 IU/L). Hematologic workup revealed a hematocrit of 27.4%, hemoglobin 9.5 g/dL and platelets 162,000/mL. The total leukocyte count was 6,900/mL with a normal differential count, but rouleaux formation was present. His serum calcium was 8.8 mg/dL and creatinine 1.2 mg/dL. Serum protein electrophoresis and immunofixation identified a monoclonal λ light-chain gammopathy. A bone marrow biopsy confirmed the relapse of myeloma, showing 70% of plasma cells, and the normal hematopoietic cells were largely replaced by sheets of tumor cells. The patient continued to receive chemotherapy for myeloma and awaited vertebroplasty.

FLOW CYTOMETRY FINDINGS

Bone marrow: CD38 69%, CD138 62%, CD38/CD138 62%, CD56 62%, CD45 7%, surface κ 2%, surface λ 1%, cytoplasmic κ 2%, cytoplasmic λ 78%.

CYTOGENETIC STUDY

Cytogenetic analysis of unstimulated cultures revealed a male karyotype with an apparently normal GTG banding pattern in all cells analyzed; the karyotype was 46, XY.

DISCUSSION

Plasma cell myeloma or MM accounts for 10% of all malignant hematologic neoplasms and about 1% of all cancer-related deaths in Western countries (1,2). In the United States, it is the most common lymphoid malignancy in blacks and the second most common in whites (3). The incidence of MM had been increased 45% during the period from 1940 through 1970, but the incidence appears to have become stable since 1992 (4).

MM is a monoclonal plasma cell neoplasm that produces monoclonal gammopathy in most cases except for the subtype of nonsecretory myeloma and some cases of solitary plasmacytoma. On the basis of clinical symptoms, MM can be divided into symptomatic myeloma and asymptomatic myeloma (5). A premalignant condition of MM is called monoclonal gammopathy of undetermined significance (MGUS) or monoclonal gammopathy, unattributed/unassociated (MG[u]), as suggested by the International Myeloma Working Group (5).

The International Myeloma Working Group suggests (i) the use of a universal standard of 3 g/dL for all classes of paraprotein and (ii) 10% clonal plasma cells in the bone marrow as the cutoff for asymptomatic (smoldering) myeloma (5). This classification does not set cutoff points for symptomatic MM, but requires the presence of end organ damage in addition to serum and/or urine paraprotein and bone marrow plasmacytosis as the diagnostic criteria. The rationale behind these criteria is that 40% of patients with symptomatic myeloma may have paraprotein <3 g/dL, and 5% of patients may have <10% plasma cells in the bone marrow (5).

On the basis of the International Myeloma Working Group classification, the World Health Organization (WHO) modified its old criteria (3) and presented a new 2008 classification (Table 6.24.1) (6). The new definition of symptomatic MM includes monoclonal protein (M-protein or paraprotein) in serum or urine, bone marrow clonal plasma cells or plasmacytoma and related organ or tissue impairment. The end organ damage is usually manifested as hypercalcemia, renal insufficiency, anemia and lytic bone lesion, which is often described with the acronym “CRAB.” However, a few patients may not have renal insufficiency. In those cases, other organ damage, such as in the liver (Fig. 6.24.1) or lung, is also acceptable for the diagnosis of symptomatic MM. In the new classification, there is no cutoff for the percentage of plasma cell in the bone marrow and the level of M-protein in serum and urine for symptomatic MM. However, 95% of patients may have more than 10% plasma cell in the bone marrow, and 30 g/L of IgG or 25 g/L of IgA in the serum. The Bence Jones protein is usually more than 1 g/24 hour of urine.

TABLE 6.24.1

WHO Diagnostic Criteria for Plasma Cell Myeloma

Symptomatic plasma cell myeloma M-protein in serum or urine Bone marrow clonal plasma cells or plasmacytoma Related organ or tissue impairment (CRAB: hypercalcemia, renal insufficiency, anemia, lytic bone lesions) Asymptomatic (smoldering) myeloma M-protein in serum at myeloma levels (>30 g/L) and/or 10% or more clonal plasma cells in bone marrow No related organ or tissue impairment (end organ damage or bone lesions [CRAB: hypercalcemia, renal insufficiency, anemia, bone lesions]) or myeloma-related symptoms MGUS M-protein in serum <30 g/L Bone marrow clonal plasma cell <10% and low level of plasma cell infiltration in a trephine biopsy No lytic bone lesions No myeloma-related organ or tissue impairment (CRAB: hypercalcemia, renal insufficiency, anemia, bone lesions) No evidence of other B-cell proliferative disorder
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Asymptomatic or smoldering MM is defined as cases with no end organ damage, osteolytic bone lesion, and myeloma symptoms (hyperviscosity, amyloidosis, or recurrent infections), but with monoclonal gammopathy and monoclonal plasma cell infiltration in the bone marrow. In those

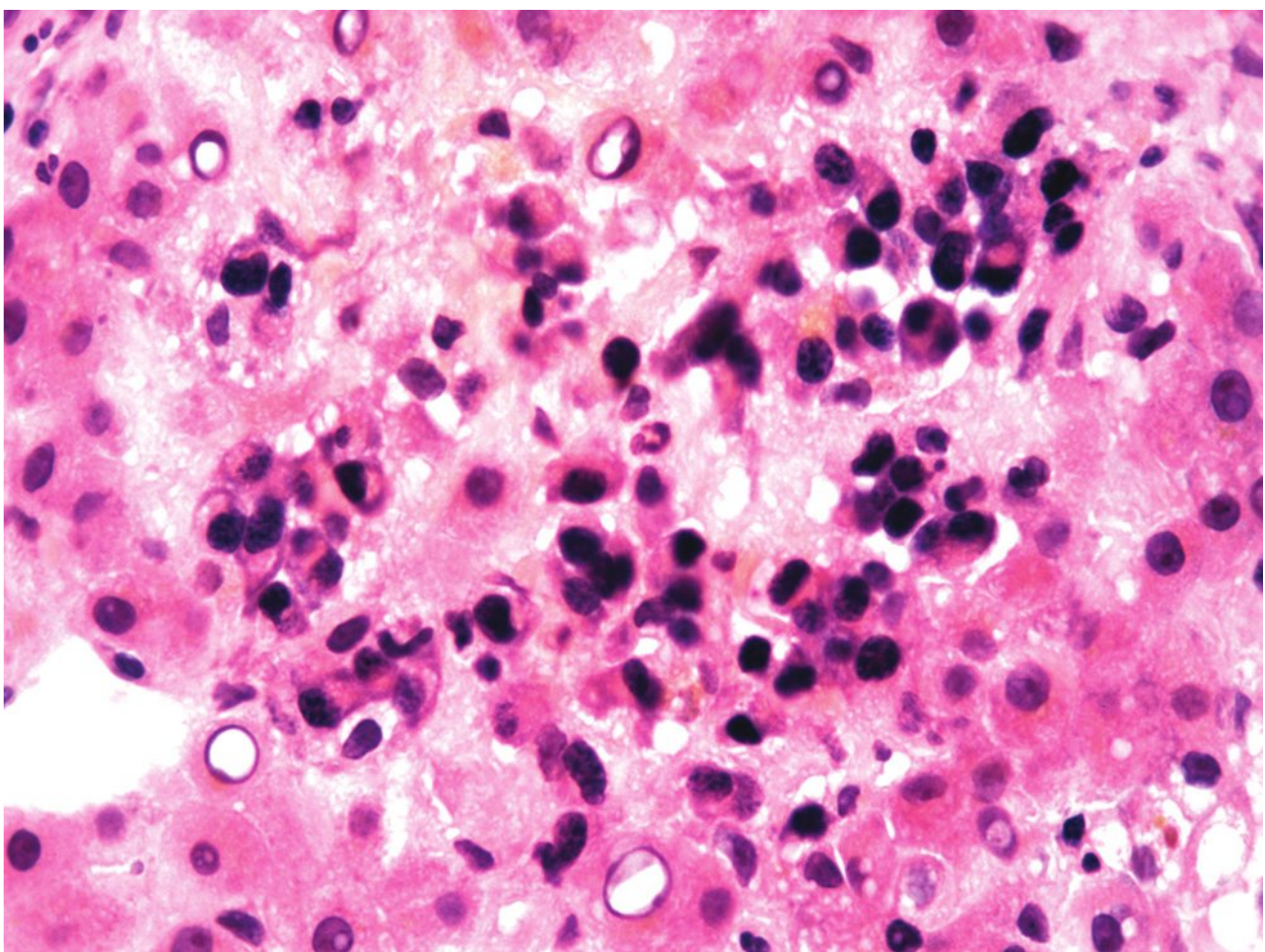


FIGURE 6.24.1 Plasma cell infiltration is demonstrated in a fine-needle liver biopsy from an MM patient. Hematoxylin and eosin, 60 × magnification.

patients, a cutoff of 10% plasma cells in the bone marrow and/or 30 g/L of M-protein in the serum are required. When the percentage of plasma cells and the M-protein level are below these cutoff points in asymptomatic patients, a diagnosis of MGUS should be made. Patients with smoldering MM or MGUS may manifest clinical symptoms, such as anemia and renal insufficiency, due to coexistence of other diseases, such as hypertension or diabetes. Those patients should not be diagnosed as symptomatic MM.

Morphology

The diagnosis of MM is usually suspected when monoclonal gammopathy in the serum (Fig. 6.24.2) and/or Bence–Jones protein in the urine are found (Figs. 6.24.3 and 6.24.4). However, as will be discussed later, several types of plasma cell dyscrasia show no or inconspicuous monoclonal gammopathy. In contrast, cases of amyloidosis may show monoclonal gammopathy, yet it is not necessarily neoplastic. Therefore, the demonstration of myeloma cells is still the most important and definitive criterion for the diagnosis of MM.

In the peripheral blood, the most striking feature is marked rouleaux formation, which is related to the quantity of paraprotein and the high erythrocyte sedimentation rate that is usually present in MM. Plasma cells are usually not seen in peripheral blood. However, a low percentage of plasma cells can be detected in 15% of MM cases. If >20% or 2,000/mL of plasma cells are present, it is designated plasma cell leukemia (PCL) (Fig. 6.24.5) (5).

The bone marrow usually shows preservation of normal hematopoietic elements, and is only partially replaced by the myeloma cells, with an average of 20% to 36% plasma cells (Fig. 6.24.6) (7). As mentioned before, the percentage of plasma cells in symptomatic myeloma cases may vary (5); therefore, the distribution pattern is a more reliable criterion for the diagnosis. Reactive plasmacytosis usually

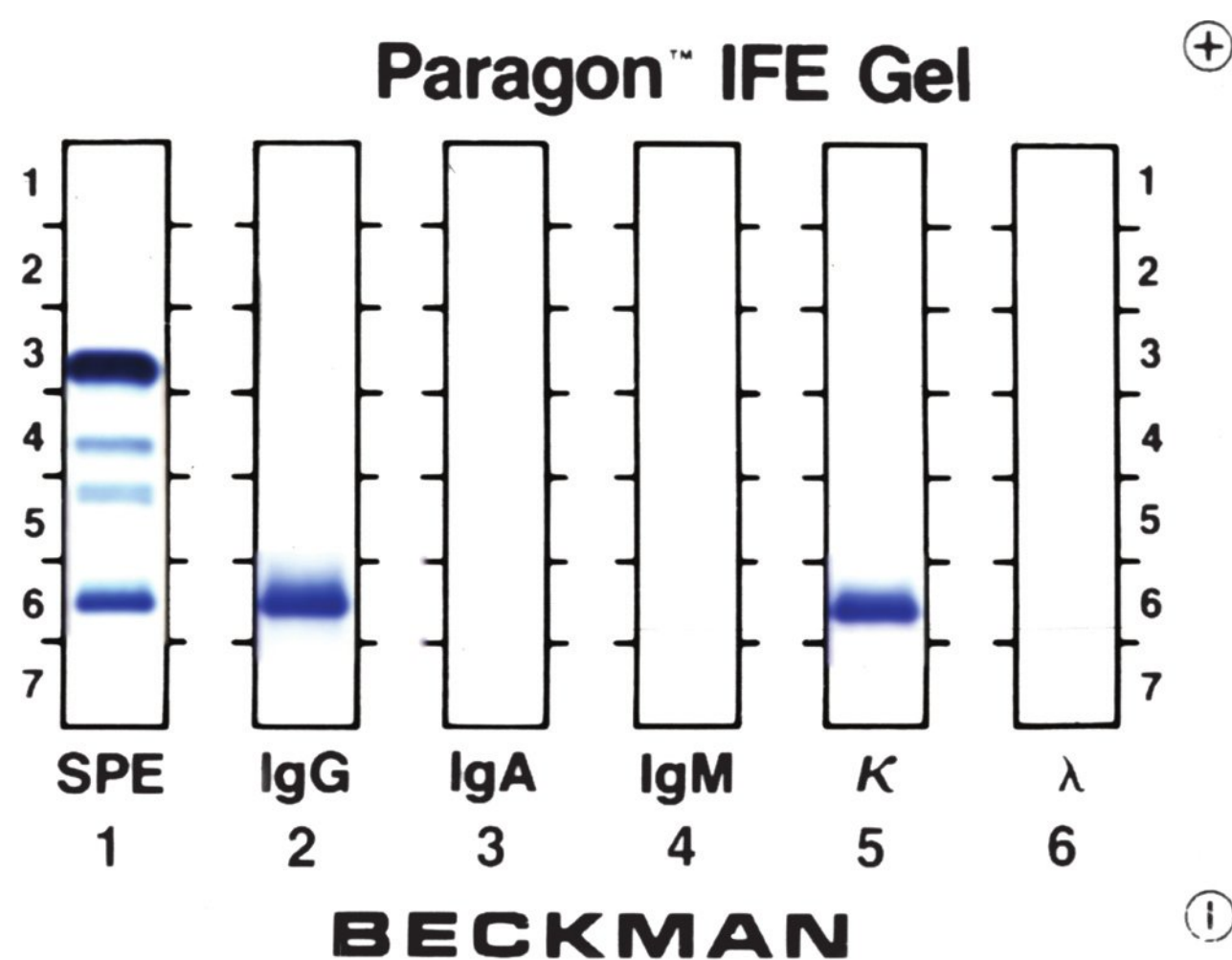


FIGURE 6.24.2 Immunofixation electrophoresis shows that the monoclonal band demonstrated in the first lane by electrophoresis reacts only to immunoglobulin (Ig) G and k antibodies, indicating monoclonal gammopathy. SPE, serum protein electrophoresis; IFE, immunofixation electrophoresis.

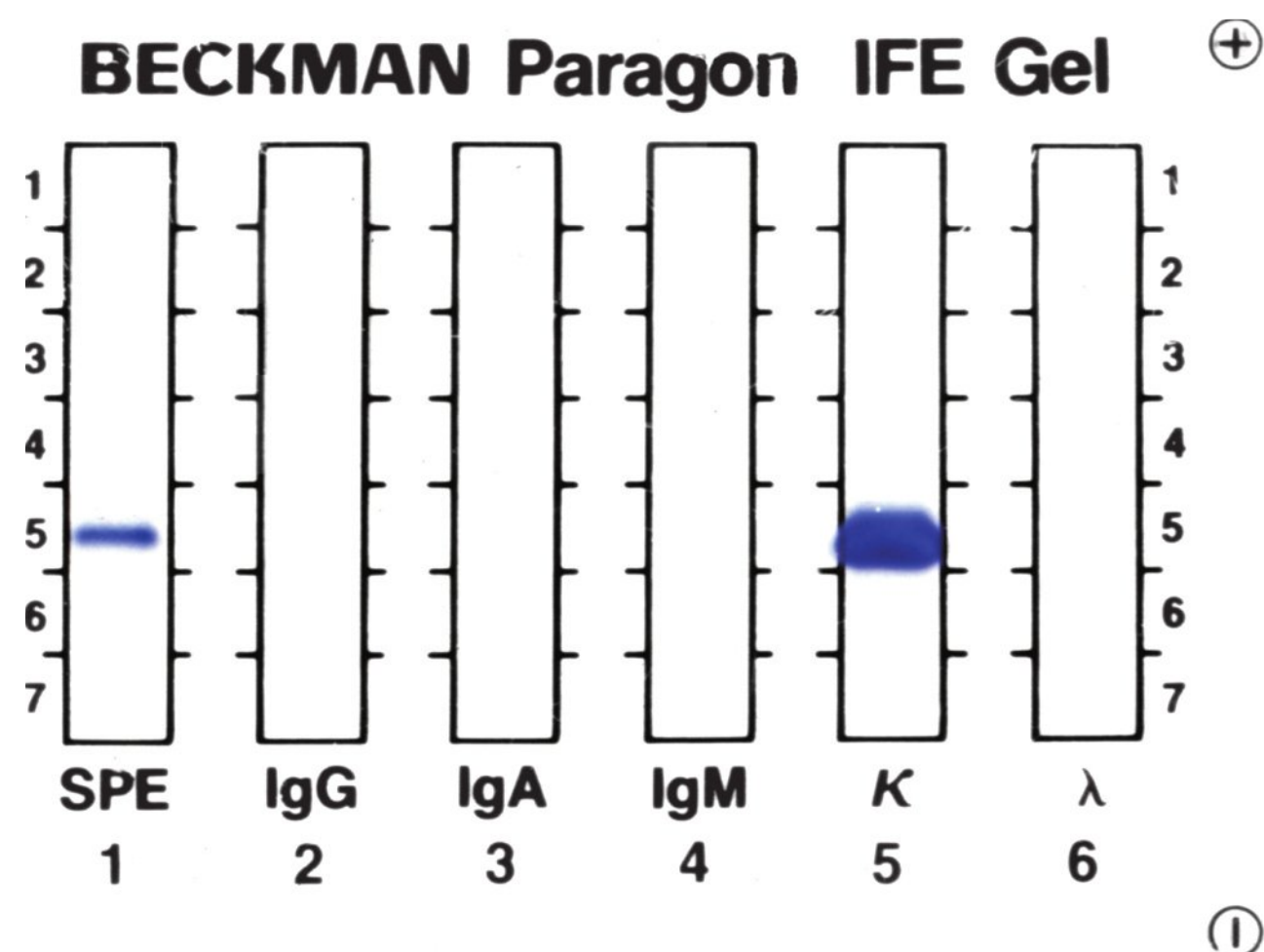


FIGURE 6.24.3 Immunoelectrophoresis shows that the monoclonal band demonstrated in urine electrophoresis (lane 1) reacts only to k light-chain antibody, consistent with Bence–Jones proteinuria. SPE, serum protein electrophoresis; IFE, immunofixation electrophoresis; Ig, immunoglobulin.

shows a cluster of 5 to 6 cells around the marrow arterioles or the so-called satellitism. In MM cases, larger clusters, nodules, or sheets of plasma cells are present randomly (mass effect) (Fig. 6.24.7) (3). Normal hematopoietic cells are usually not seen in the “tumor mass.” In patients with radiologic evidence of osteolytic bone lesion, osteoclastic activity may be demonstrated histologically (Fig. 6.24.8).

The morphology of the myeloma cells in most cases is similar to that of normal plasma cells. In other words, the myeloma cells show an eccentric nucleus with a perinuclear hof and deep blue cytoplasm. The nucleus contains

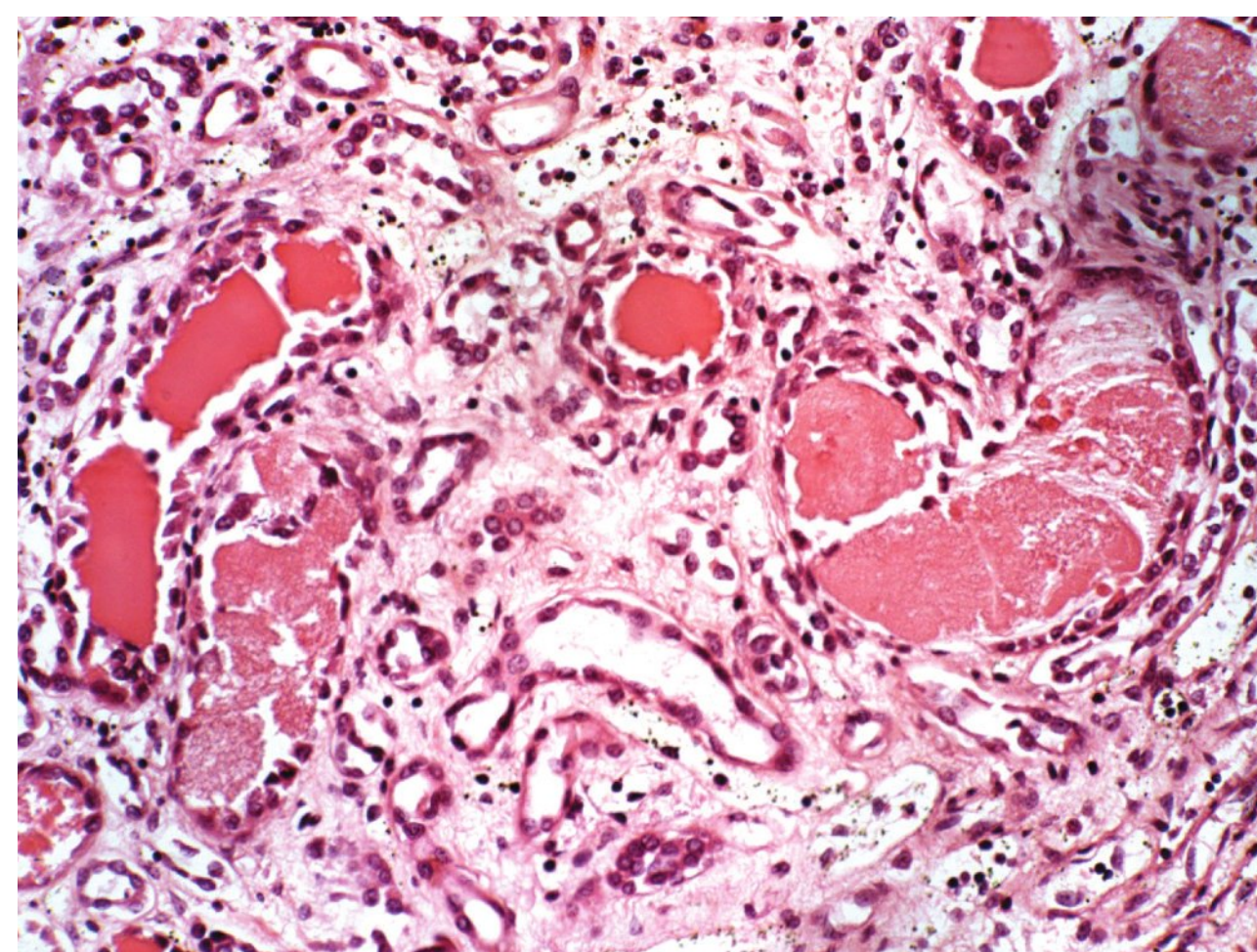


FIGURE 6.24.4 Kidney section from an autopsy case of MM shows Bence–Jones protein casts inside the renal tubules. Hematoxylin and eosin, 20 × magnification.

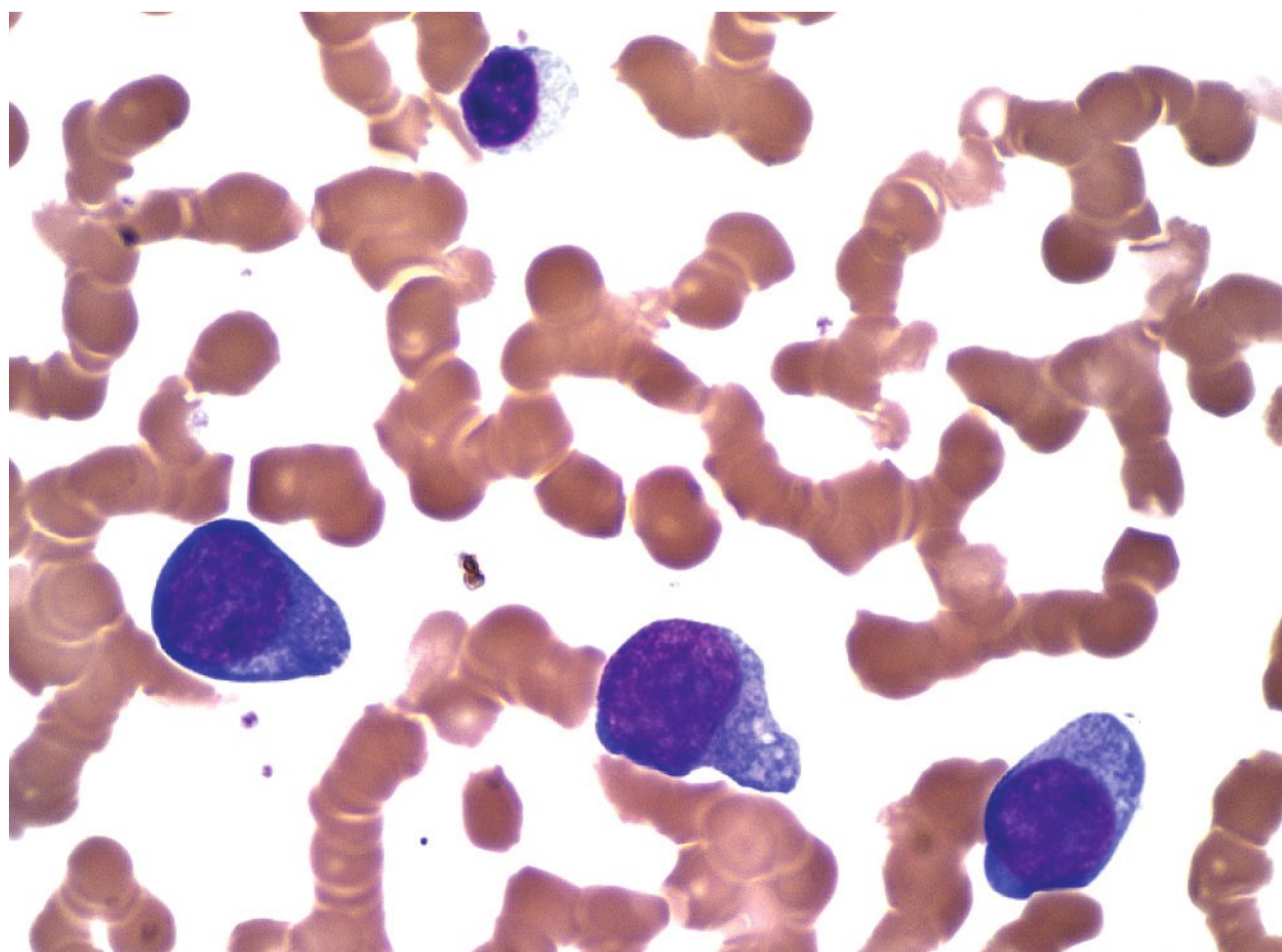


FIGURE 6.24.5 Peripheral blood smear from a case of plasma cell leukemia shows three plasmablasts and one plasma cell with prominent rouleaux formation in the background. Wright–Giemsa, 100 × magnification.

a clock face or cartwheel chromatin pattern. However, in some cases of MM, the tumor cells are poorly differentiated or undifferentiated, for which immunologic identification becomes necessary.

On the basis of differentiation, MM can be classified into mature, intermediate, immature, or plasmablastic cytologic type (7). The plasmablastic type shows the worst prognosis, with a median survival of 10 months compared with 35 months for the other types. However, for the other three types, there appears to be no significant difference in survival (7). MM can also be divided into poorly differentiated, polymorphous, asynchronous, or blastic types (8). A more recent morphologic classification divides MM cells into mature, immature, plasmablastic, anaplastic, and (rare) variants (9). When the plasma cells show equal numbers of mature and immature forms, it is classified as intermediate type. A small lymphocyte-like plasma cell

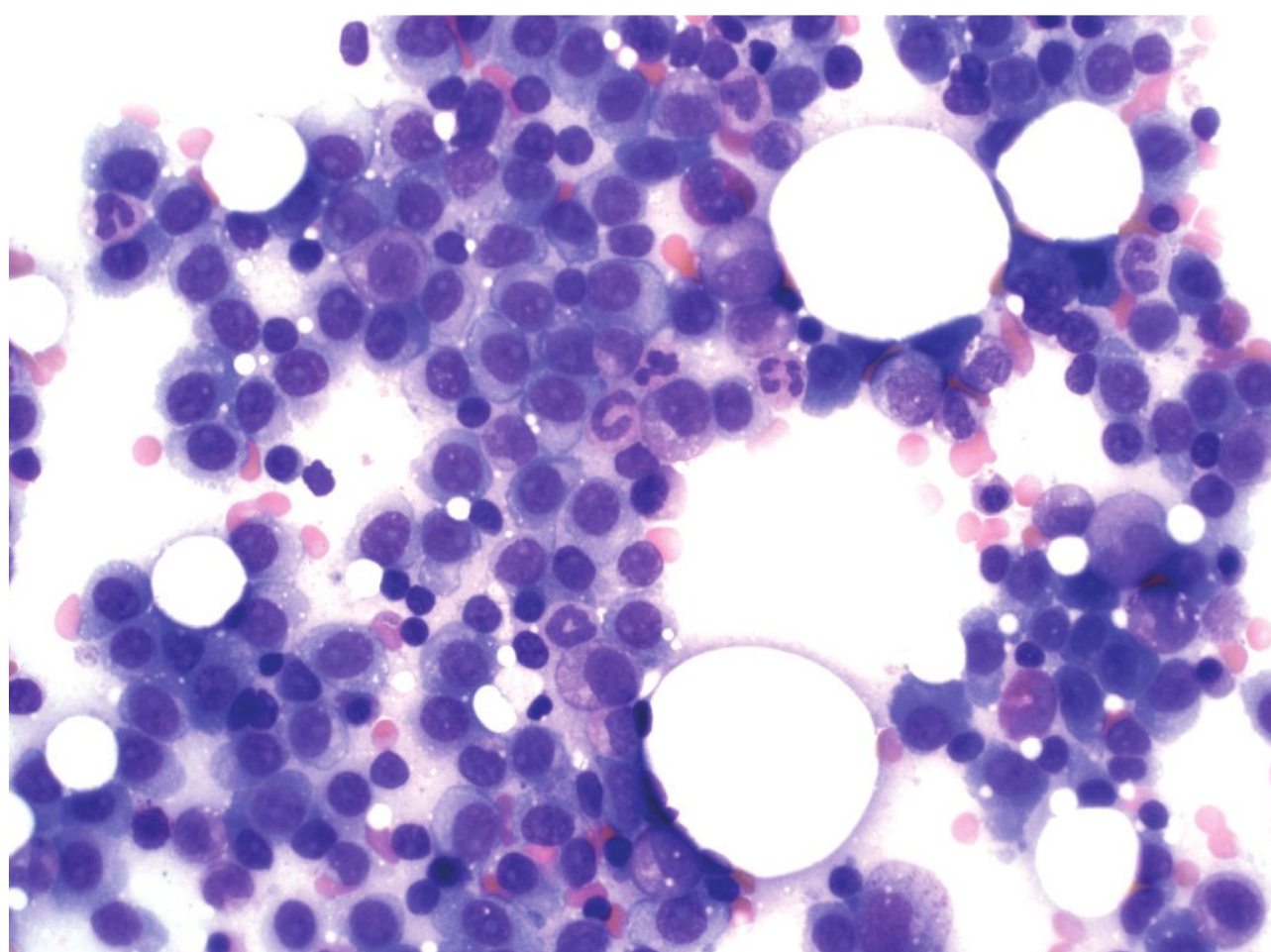


FIGURE 6.24.6 Bone marrow aspirate shows clusters of myeloma cells. Wright–Giemsa, 40 × magnification.

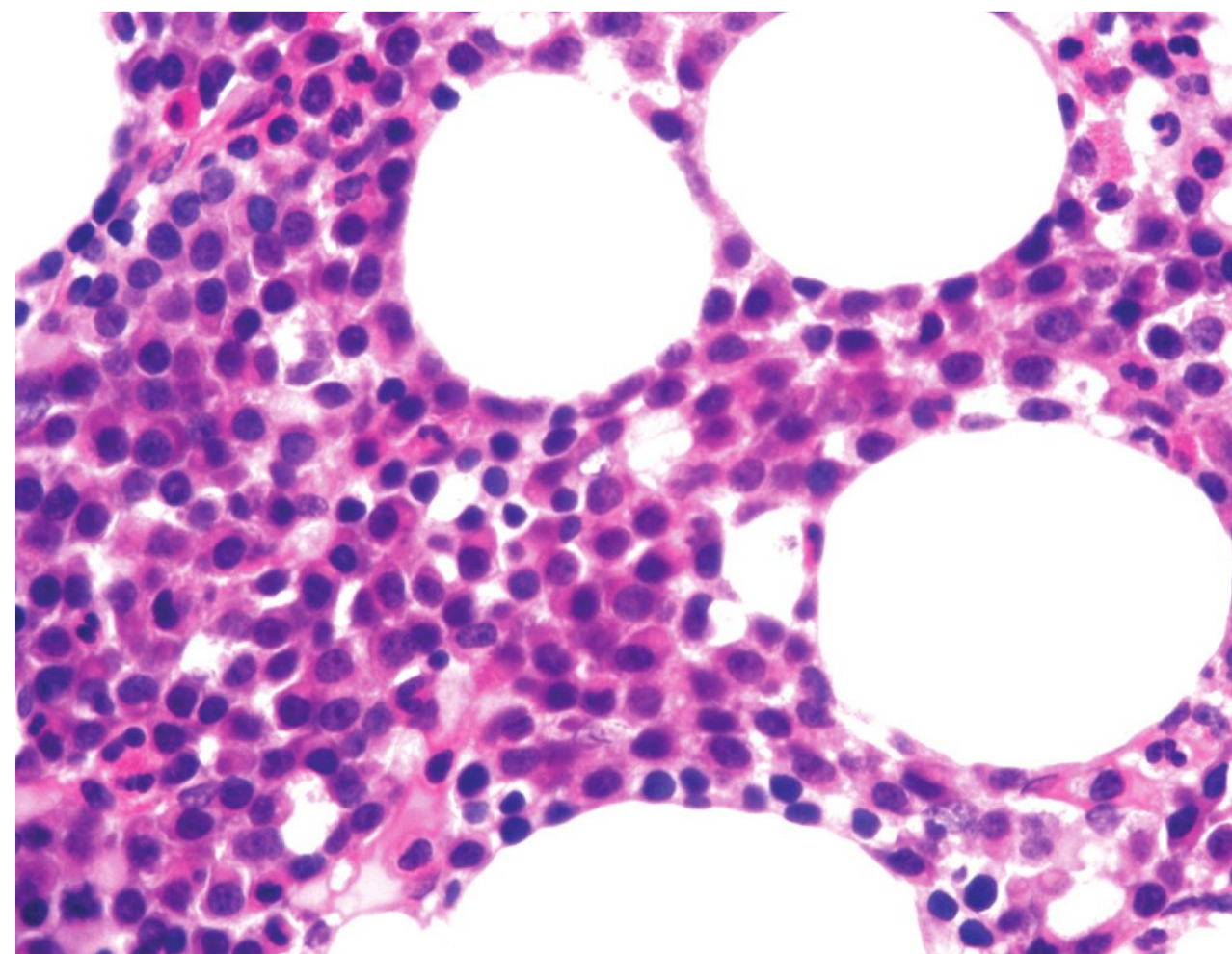


FIGURE 6.24.7 Bone marrow biopsy shows sheets of myeloma cells. Hematoxylin and eosin, 60× magnification.

myeloma may contain large numbers of lymphocytes that mimics small lymphocytoid lymphomas, which can only be differentiated by immunophenotyping (10).

The immaturity of myeloma cells can be manifested as larger nucleus, less condensed chromatin, and presence of nucleoli. The cytoplasm of myeloma cells may show fraying borders, cytoplasmic shedding, and the presence of vacuoles, granules, and hyaline and crystalline inclusions (7). The term “Russell bodies” refers to intracytoplasmic cherry-red refractive round inclusions that may help recognize the myeloma cells (Fig. 6.24.9). However, Russell bodies also can be seen in normal, actively secreting plasma cells. When there are multiple pale blue-white grape-like cytoplasmic inclusions present, the plasma cells are called Mott or Morula cells (Fig. 6.24.10).

When the inclusion body is present in the nucleus, it is called a Dutcher body (Figs. 6.24.11 and 6.24.12), which

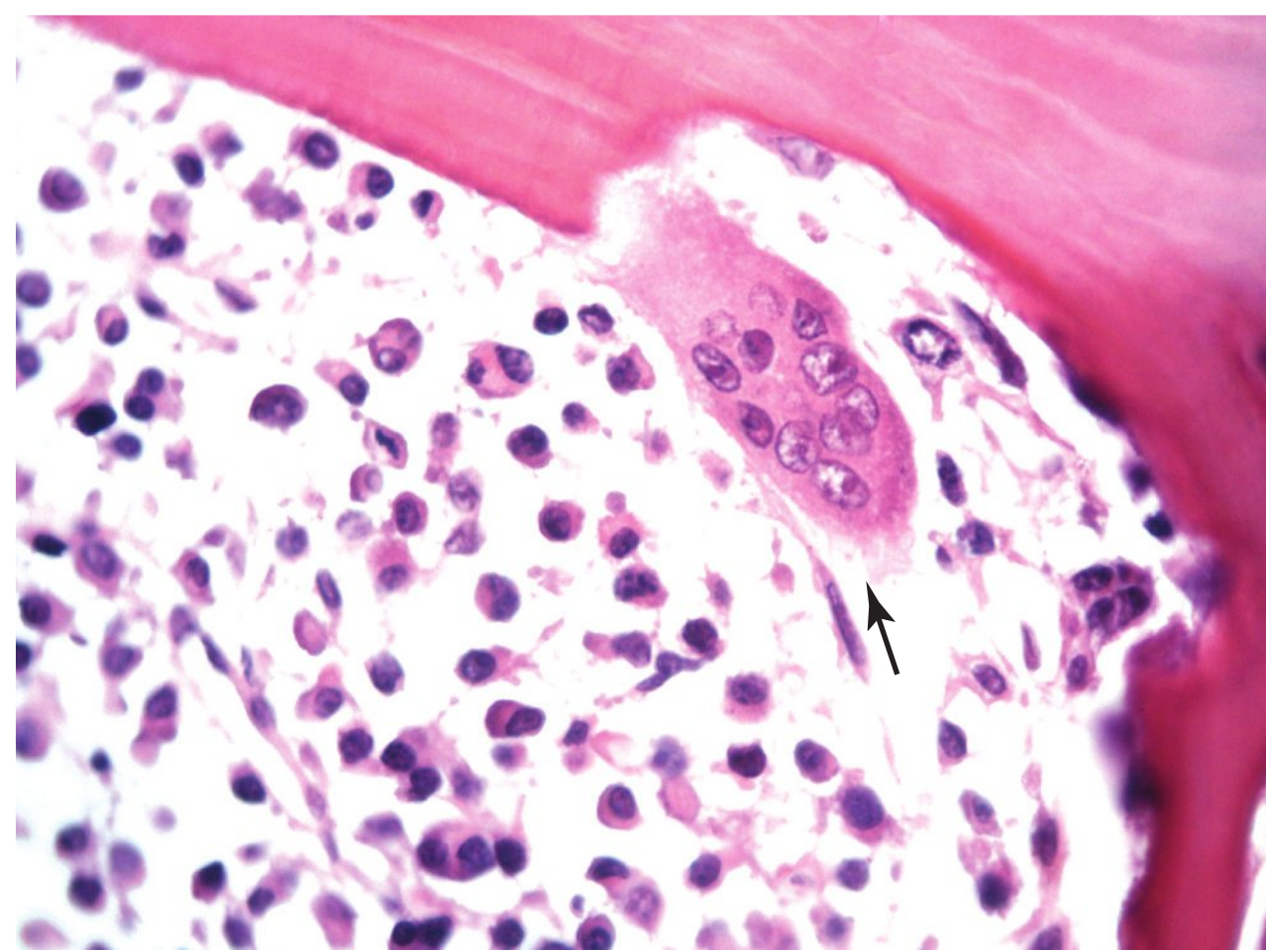


FIGURE 6.24.8 Bone marrow biopsy shows osteoclastic lesion with an osteoclast (arrow). Hematoxylin and eosin, 60 × magnification.

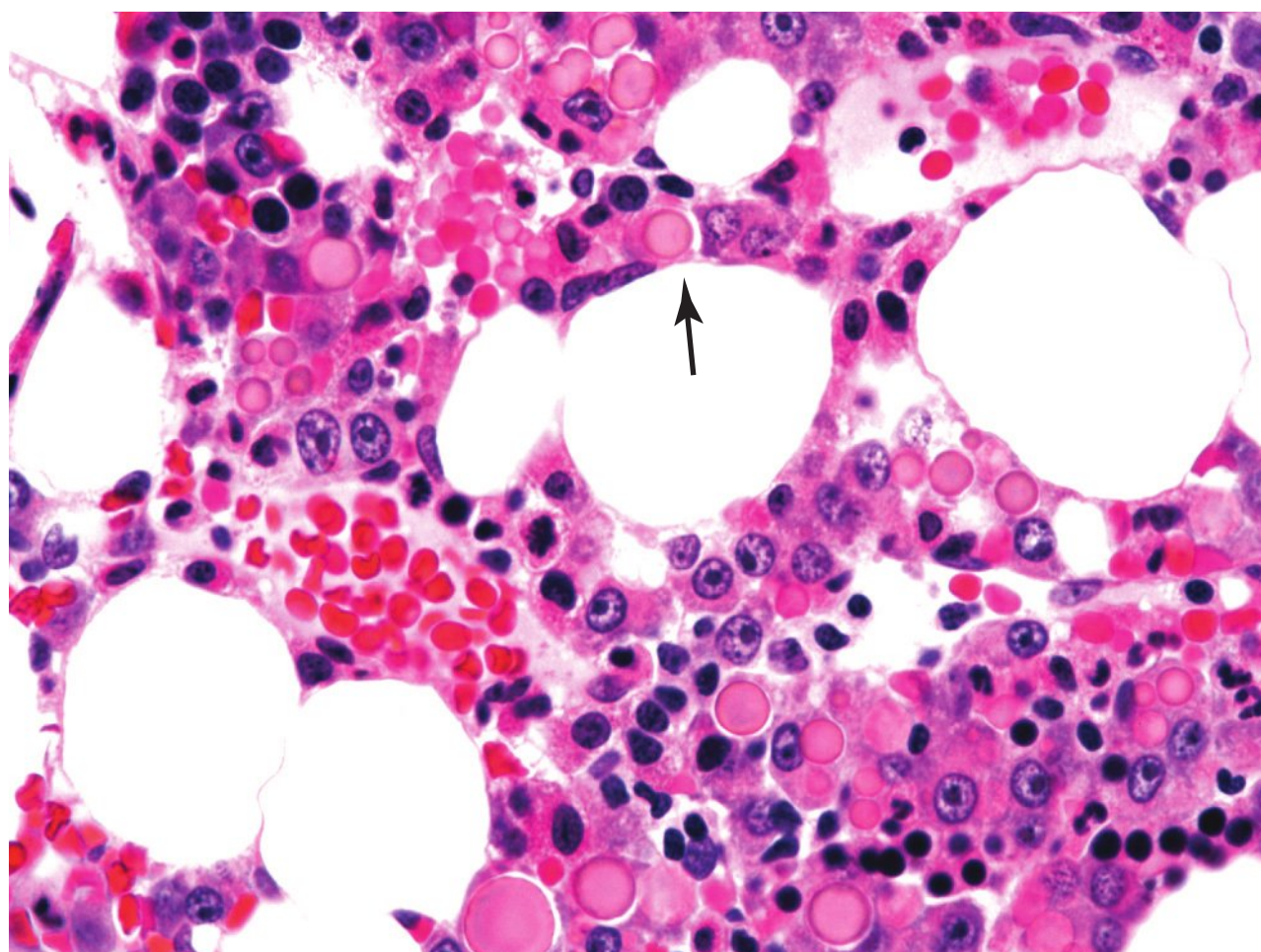


FIGURE 6.24.9 Bone marrow aspirate shows multiple Russell bodies (arrow) inside and outside of myeloma cells. Wright-Giemsa, 100 × magnification.

is seldom seen in normal plasma cells and is thus helpful for the diagnosis. Both Russell and Dutcher bodies are positive for periodic acid-Schiff stain. Cytoplasmic crystalline inclusions (Fig. 6.24.13) are commonly seen in myeloma patients with the adult Fanconi syndrome (7). Multinucleation and red (flaming) cytoplasm are characteristic of a small number of IgA myeloma. Gaucher-like cells or thesaurocytes that contain fibrillar structures can be seen in a few cases (3).

Because the plasmablastic type of MM carries a particularly poor prognosis, it is important to define the morphology of the plasmablasts (Fig. 6.24.5). According to Rajkumar and Greipp (2), four criteria identify plasmablasts: (i) presence of a fine reticular nuclear chromatin pattern with minimal or no chromatin clumping, (ii) large nuclear size (estimated to be >10 nm), (iii) cytoplasm with little or no perinuclear hof region, and (iv) less abundant

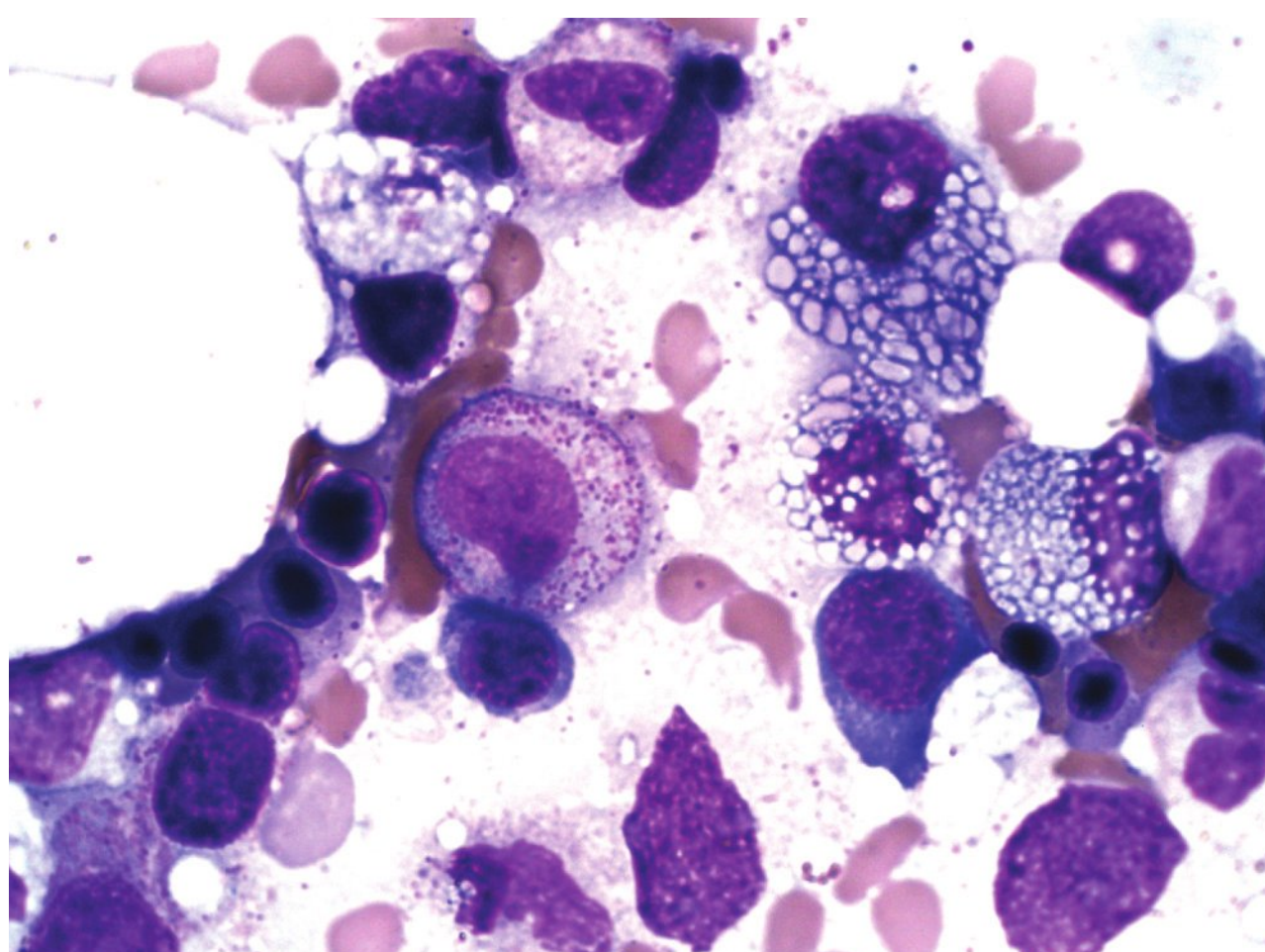


FIGURE 6.24.10 Bone marrow biopsy shows several myeloma cells (Mott cells) containing hyaline cytoplasmic inclusions. Hematoxylin and eosin, 60 × magnification.

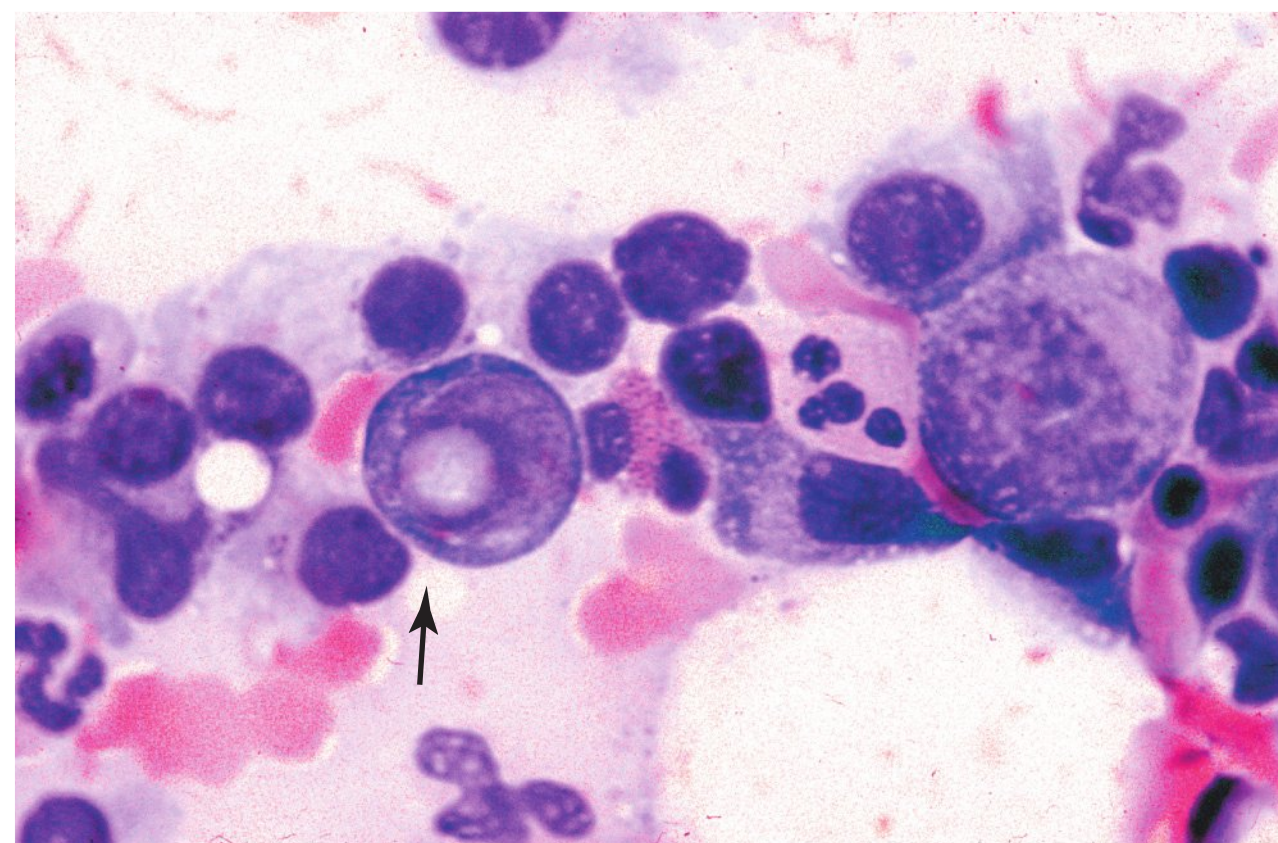


FIGURE 6.24.11 Bone marrow aspirate shows two plasmablasts, one with a huge intranuclear inclusion (Dutcher body) (arrow). Wright-Giemsa, 100 × magnification.

cytoplasm comprising less than one half the nuclear area (Fig. 6.24.14).

The following variants of MM have no or only small amount of paraprotein demonstrated in serum and/or urine.

Nonsecretory myeloma is present in 1% to 5% of all patients with MM. It has all characteristics of MM except that paraprotein is absent in both serum and urine (3,5,6,11). However, cytoplasmic Ig can be identified with immunocytologic techniques in about 85% of cases. This variant has a lower percentage of plasma cells in the bone marrow and less depressions of normal Ig (3). In some cases of nonsecretory myeloma, paraprotein can be demonstrated by serum-free light-chain (sFLC) measurement; those cases are designated oligosecretory myeloma (12,13).

Solitary plasmacytoma of bone is present in 5% of patients with MM and is characterized by the presence of only one focus of monoclonal plasmacytosis in the bone

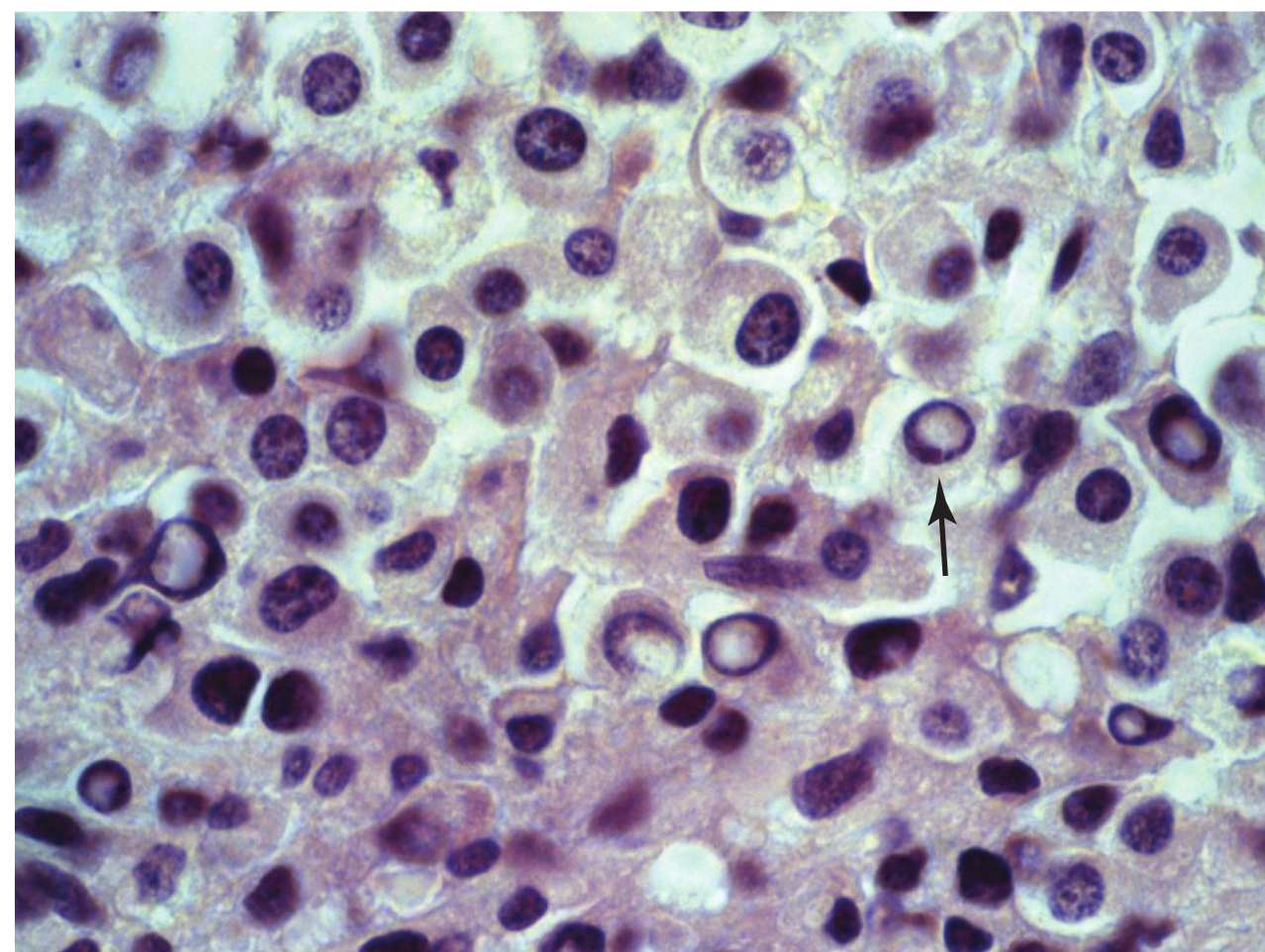


FIGURE 6.24.12 Bone marrow biopsy shows many myeloma cells containing Dutcher bodies (arrow). Hematoxylin and eosin, 100 × magnification.

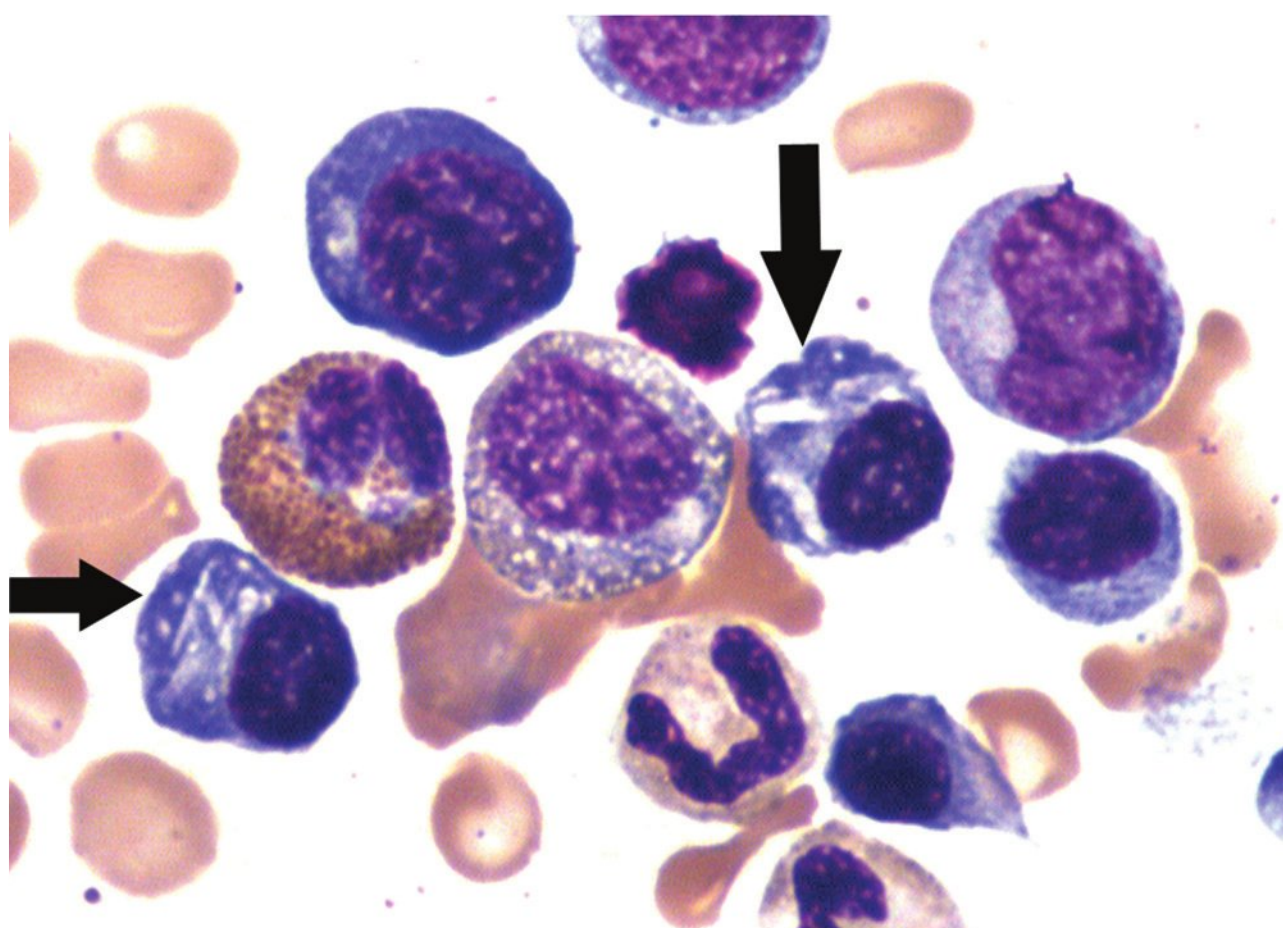


FIGURE 6.24.13 Crystalline inclusions are seen in two plasma cells (arrows) from a bone marrow aspirate. Wright–Giemsa, 200 × magnification.

marrow as detected by radiological examination and confirmed with biopsy (Fig. 6.24.15) (3,5,6,14). A large proportion of patients with solitary plasmacytoma of bone fail to show a monoclonal paraprotein even on immunofixation studies. In the minority of patients, low-level gammopathy can be demonstrated. However, with the sFLC test, paraprotein can be detected in most patients (15). Local radiation of the solitary lesion usually leads to the disappearance of paraprotein. The persistence of paraprotein after radiation is associated with an increased risk of progression. Almost 50% of patients with this variant progress to overt myeloma (3).

Extramedullary plasmacytoma involves the soft tissue, most frequently the upper respiratory tract, including the nasal cavity and sinuses, nasopharynx, and larynx (Fig. 6.24.16) (3,5,6,14). Some cases involving the gastrointestinal tract may represent extranodal marginal zone B-cell lymphoma with extreme plasma cell differentiation

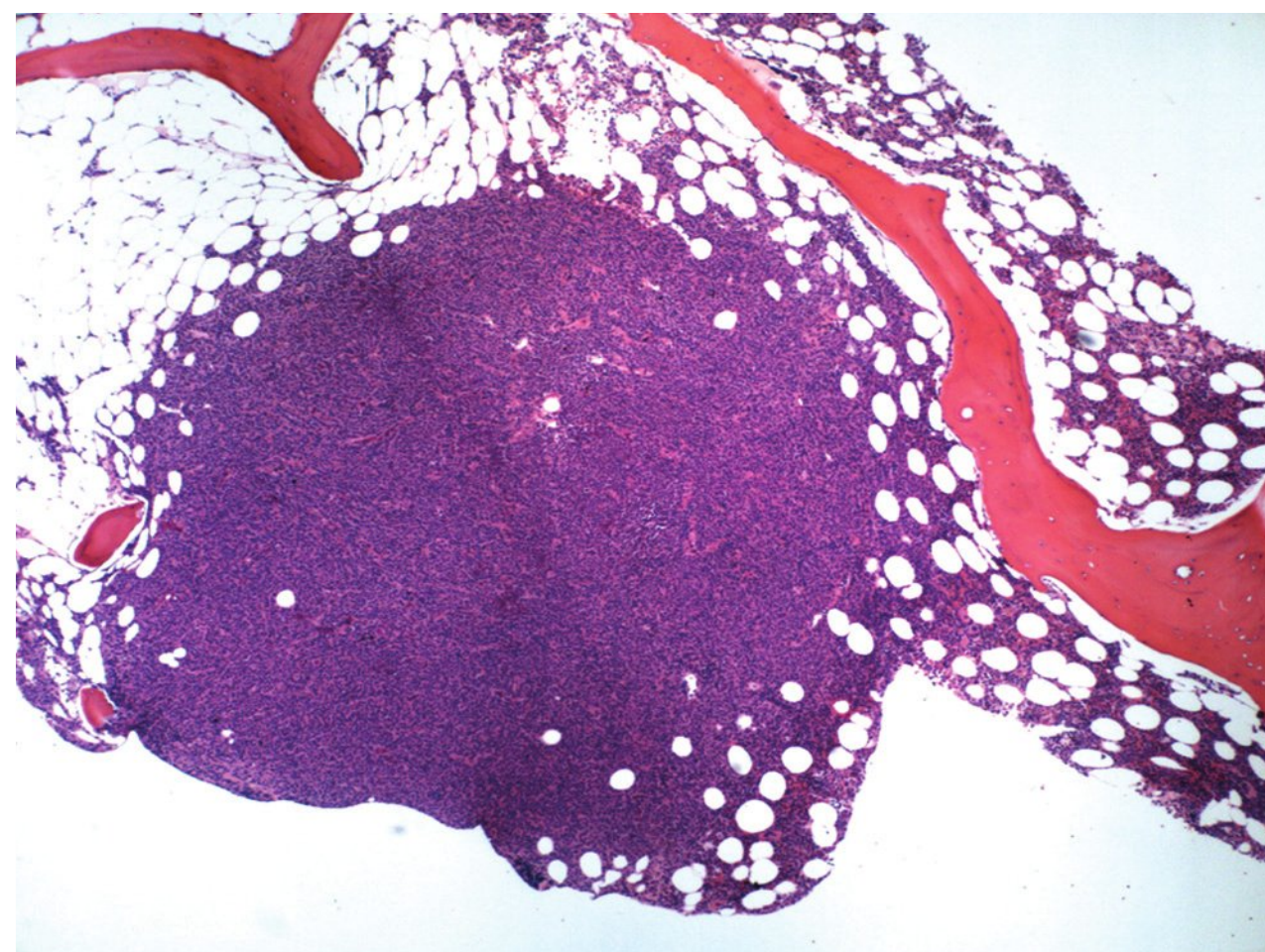


FIGURE 6.24.15 A solitary plasmacytoma is demonstrated in a bone marrow biopsy. Hematoxylin and eosin, 4 × magnification.

(3). A monoclonal protein, predominantly IgA, is demonstrated in only 25% of this variant. Extramedullary plasmacytoma may recur in multiple sites including the soft tissue and bone. This condition is called multiple solitary plasmacytoma (5). The conversion rate of extramedullary plasmacytoma to overt MM is approximately 15% (5).

In cases of the above three entities, the most reliable diagnostic techniques are immunohistochemical, immunocytochemical, and/or immunofluorescent staining to identify a monoclonal κ or λ protein in the tumor cells. However, the sFLC test may also help to substantiate the diagnosis. Flow cytometry can also be used for the diagnosis of these entities and is discussed later.

Immunophenotype

MM is usually detected by the presence of monoclonal gammopathy in the serum and/or urine or by the detection

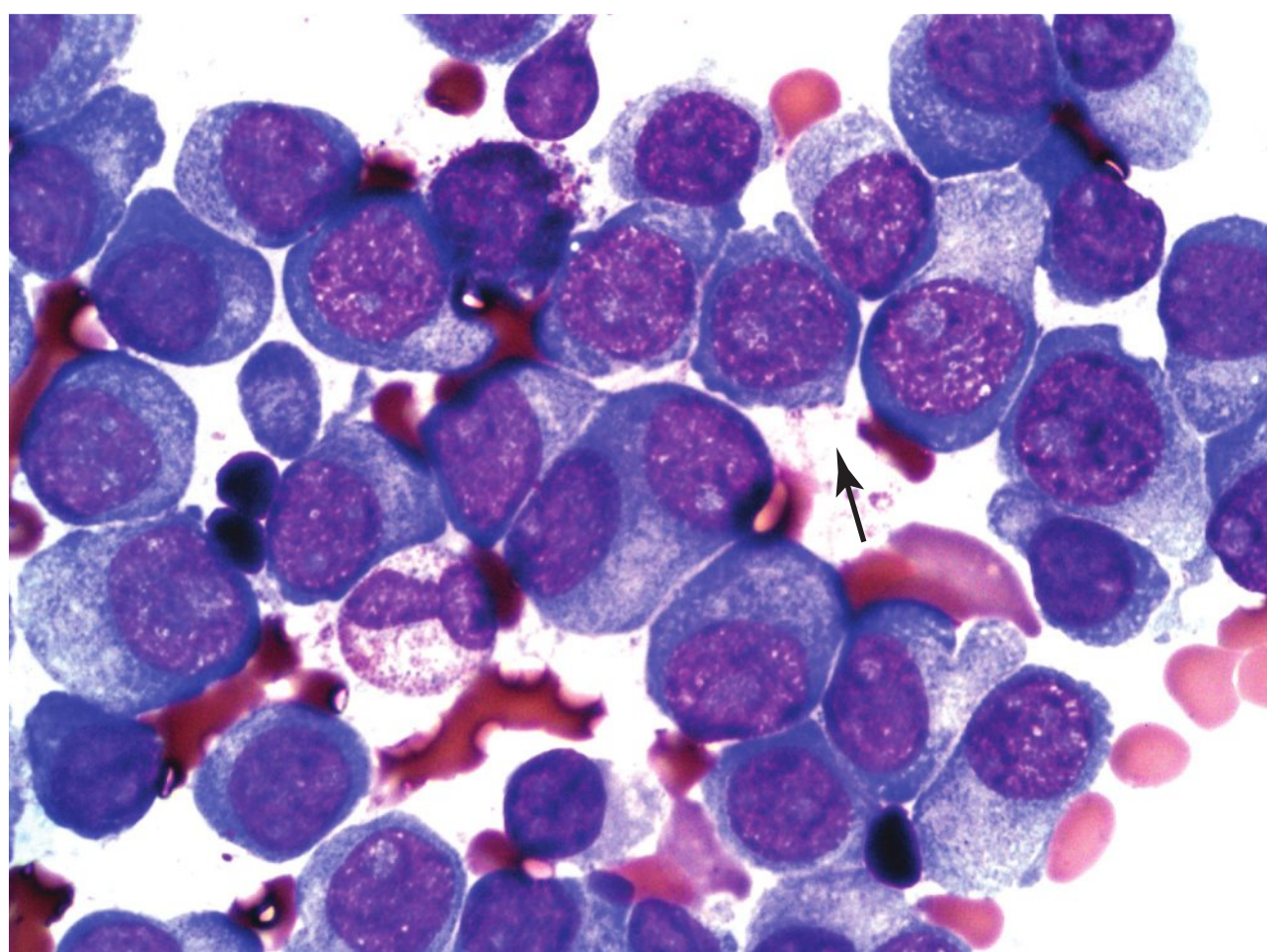


FIGURE 6.24.14 Bone marrow aspirate shows several plasmablasts (arrow). Wright–Giemsa, 100 × magnification.

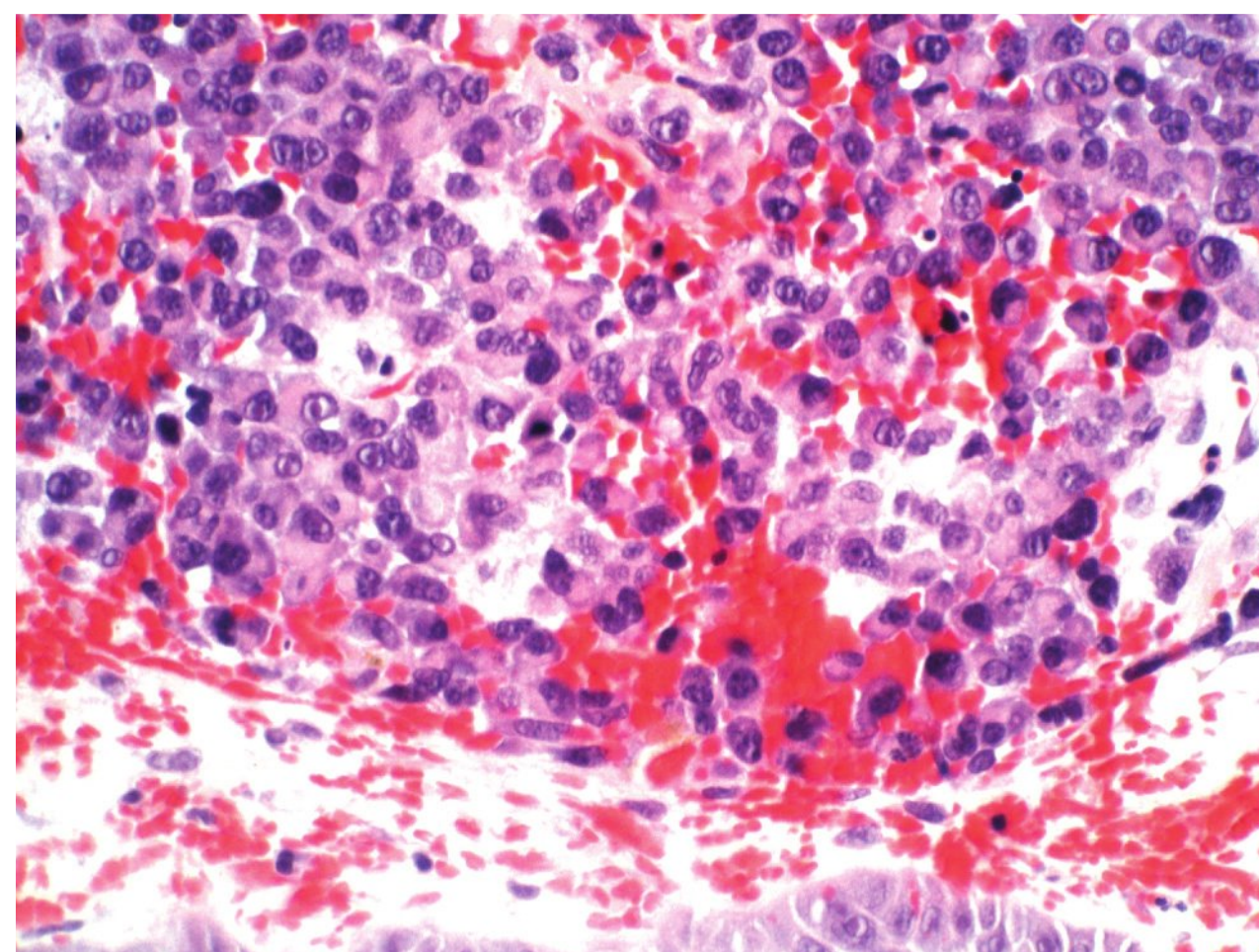


FIGURE 6.24.16 Plasmacytoma from the nasopharynx. Hematoxylin and eosin, 40 × magnification.

of osteolytic bone lesions that are further confirmed by bone marrow biopsy. Bone marrow biopsy is performed not only for diagnosis but also for the evaluation of the tumor mass, which is an important predictor for prognosis.

The most important immunophenotype is the presence of monoclonal cytoplasmic Ig and the absence of surface Ig and B-cell antigens (CD19, CD20, and CD22) (Fig. 6.24.17) (16). When surface Ig is present simultaneously with cytoplasmic Ig, it is usually seen in Waldenström macroglobulinemia and/or lymphoplasmacytic lymphoma; otherwise, it can be an artifact due to the presence of cytophilic Ig on the surface of the tumor cells. The only positive surface B-cell marker is CD79a, which is an Ig-associated pan-B-cell antigen (17). Those cases with positive CD20 and surface Ig usually have a poor prognosis (2). CD10 is also considered a poor prognostic predictor by some, but conflicting results have been reported by others (2).

In immunohistochemical staining, the demonstration of the predominance of k or l light-chain or a monoclonal k/l ratio is most important for the diagnosis. However, when there is coexistence of large numbers of normal plasma cells and macrophages with MM cells, the k/l ratio may not be clear cut and other markers may be required to clarify the situation.

Previously, an immunophenotype of positive CD38, negative HLA-DR, and negative or weakly positive CD45 is considered most specific for MM (18). However, the discovery of two relatively new markers makes the diagnosis much more accurate. The first one is CD56: The presence of this marker distinguishes MM from reactive plasmacytosis, as CD56 is negative in normal plasma cells (19,20). Several studies advocated the use of CD56 and CD19 to distinguish normal from neoplastic plasma cells (20–22). Normal plasma cells are CD19+, CD56-, whereas MM cells are CD19-, CD56+. In the minority of MM cases, CD56 can be negative, and CD19 can be positive. In cases of MGUS, one report showed no CD56+ cells in 23 patients studied (19), but another study revealed the coexistence of both CD56+ and CD56- cells in all five patients (21).

The second new marker is CD138, which is a collagen-1-binding proteoglycan, also known as syndecan (22,23). Because of its collagen binding nature, plasma cells may occasionally become negative in fibrotic area as CD138 is shed from the surface membrane into the surrounding fibrotic matrix (24). CD138 can also help to distinguish normal plasma cells (CD19+, CD38+, CD56-, CD138+) from B-progenitor cells (CD19+, CD38+, CD56-, CD138-). Therefore, a CD38/CD138 gating provides the best separation of plasma cells from other leukocytes (20).

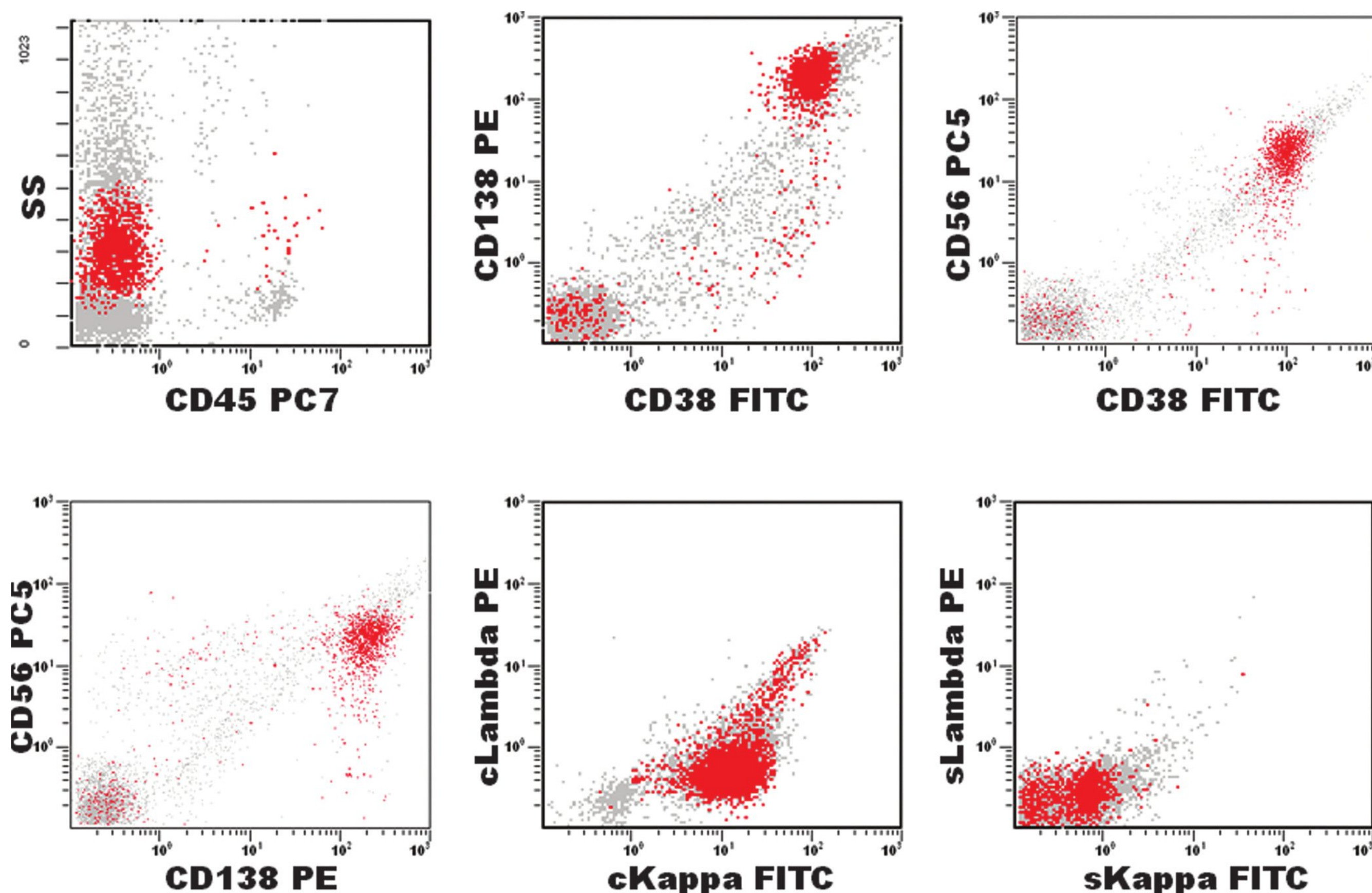


FIGURE 6.24.17 Flow cytometric histograms shows dual staining of CD38/CD138, CD38/CD56, CD138/CD56, and monoclonal cytoplasmic k. No surface immunoglobulins are demonstrated. FITC, fluorescein isothiocyanate; PC5, phycoerythrin-cyanin 5; PC7, phycoerythrin-cyanin 7; PE, phycoerythrin; c, cytoplasmic; s, surface.

Primary PCL differs from MM in the expression of CD20 and frequent absence of CD56, CD117, and HLA-DR. The last three antigens are often demonstrated in MM (25).

Other markers that are positive for plasma cells include plasma cell-associated antigen (PCA)-1, PC-1, CD24, CD28, CD30, CD31, CD40, CD44 (homing-associated cell-adhesion molecule), and CD54 (intracellular adhesion molecule 1) (2,16,26). In a study of 49 MM patients, bcl-2 was demonstrated in the tumor cells of 97% cases (27). However, bcl-2 is also present in normal plasma cells. Ki-67 can be expressed in MM cells (particularly those with plasmablastic morphology), but it is not present on normal plasma cells (27). It is associated with higher B₂M and with advanced or relapsed disease.

In addition, myeloma cells may also show T cell, myelomonocytic (CD11b, CD11c, CD13, CD33), megakaryocytic (CD41), and/or erythroid (glycophorin A) antigens. The presence of markers of multiple cell lineages suggests that MM is an early hematopoietic stem cell disorder manifesting itself at the mature stage of B-cell lineage, analogous to the situation in chronic myeloid leukemia (28–30).

Myeloma cell-associated antigen has also been used to identify circulating lymphocytes in MM cases (31). If the circulating cells carry CD38 or PCA-1, the prognosis is poor. These lymphocytes are considered the precursors of the myeloma cells and can also be identified with idiotypic surface Ig, CD10, DNA aneuploidy, or Ig gene rearrangement (28).

Comparison of Flow Cytometry and Immunohistochemistry

Under most circumstances, MM can be diagnosed on a morphologic basis. When the bone marrow infiltration is limited or the tumor cells are poorly differentiated, immunohistochemical staining for k and l Igs is needed. A predominantly k or l light-chain staining should be enough to make the diagnosis unless lymphoma is suspected. When Ig staining is inconclusive, CD38 or CD138 stain may help to highlight the plasma cells. CD56 reaction will confirm the malignant nature of the infiltrate. MM cells are also positive for CD79a and VS38c (6). Ki-67 not only identifies the neoplastic plasma cells; its presence in high percentage also indicates a high-grade tumor, for example, the plasmablastic form.

Flow cytometry may also demonstrate all of the above markers except VS38c, and Ki-67. The major advantage of flow cytometry is to distinguish cytoplasmic versus surface Ig staining. A monoclonal cytoplasmic Ig pattern can be seen in MM, and a monoclonal surface Ig pattern is seen in various types of non-Hodgkin lymphoma. The coexistence of both monoclonal cytoplasmic and surface Ig is demonstrated in lymphoplasmacytic lymphoma.

The current case had the history of bone pain, and lytic bone lesion was demonstrated by radiology. Flow cytometry revealed a very characteristic immunophenotype with the presence of CD38/CD138, CD56, and a monoclonal cytoplasmic Ig pattern. The negative CD45 and surface Igs were also important findings. However, the patient showed low levels of IgG, IgA, and IgM and no quantitation of Ig light chains. Although immunofixation and flow cytometry demonstrated monoclonal l light chain in this case,

a quantitation of light chain should have been performed as this would be useful for therapeutic monitoring. In addition, as no monoclonal heavy chain was demonstrated, IgD and IgE quantitation or immunofixation should have been performed to exclude IgD or IgE myeloma. Light-chain disease usually causes severe renal damage; therefore, it is unusual to see that the patient had a normal creatinine level at the late stage of the disease, as evidenced by the presence of 70% plasma cells in the bone marrow.

Molecular Genetics

In early studies, most MM cases were found to be diploid. However, with the advances of modern technology, almost all cases of MM show aneuploidy (1,32). Among the cases with cytogenetic abnormalities, 65% are hyperdiploid, 20% hypodiploid, and 15% pseudodiploid (33).

In MM and MGUS, there appear to be two pathways of pathogenesis. Nonhyperdiploid tumors have a very high incidence of IgH translocations involving five recurrent partners and a relatively high incidence of chromosome 13/13q14 loss (4,32). Hyperdiploid tumors are more frequently associated with multiple trisomies involving chromosomes 3, 5, 7, 11, 15, 19, and 21.

The five recurrent partners in IgH translocation include 11q13 (cyclin D1), 6p21 (cyclin D3), 4p16 (fibroblast growth factor receptor 3 and multiple myeloma SET domain), 16q23 (c-maf), and 20q11 (mafB) (4). These chromosome translocations may lead directly (11q13-cyclin D1 and 6p21-cyclin D3) or indirectly (4p16, 16q23, cyclin D2) to cyclin D dysregulation. In hyperdiploid tumors, cyclin D1 or, less frequently, cyclin D2 is dysregulated by an undefined mechanism. The dysregulation of cyclin D genes may render the tumor cells more susceptible to proliferative stimuli through interleukin-6 (IL-6), vascular endothelial growth factor, or other cytokines produced by bone marrow stromal cells (4).

One of the most common translocations in MM is t(11;14)(q13;q32), which is also found in mantle cell lymphoma. However, the translocation in MM takes place at a g switch region, and that in mantle cell lymphoma is in or near a JH segment (34). Patients with this translocation usually have a poor prognosis (32).

The presence of deletion or loss of chromosome 13 has been associated with poor prognosis in MM patients. Initially, it was assumed that deletion of the retinoblastoma gene (Rb) is responsible for the adverse effect. However, Rb protein level is not affected in those patients with -13q; therefore, other genes (i.e., DBM, BRCA2) may play a more critical role in the prognosis (33). Nevertheless, disruption of the Rb pathway by inactivation of Rb or p18NK4c can occur in a low frequency in the late stage of the disease (4).

The deletion of the long arm of chromosome 17 (17q-) is also associated with poor prognosis in MM patients. This is directly related to the deletion of a tumor-suppressor gene, p53 (35). Point mutations of p53 have been found more frequently in extramedullary relapse of myeloma (1). The t(4;14)(p16.3;q32) translocation is associated with IgA isotype, aggressive features, and an unfavorable prognosis (36).

Several additional oncogenes may be involved in the progress of MM. These include the de-regulation of c-myc, which is seen in the initial phase of MM, and point

TABLE 6.24.2

Salient Features for Laboratory Diagnosis of Plasma Cell Myeloma

1. Presence of clusters of plasma cells in bone marrow
2. Presence of monoclonal gammopathy
3. Progressive elevation of paraprotein with background Ig suppression
4. Presence of Bence Jones Proteinuria
5. Monoclonal cytoplasmic Ig (k or l predominance), CD79a, VS38c demonstrated by immunohistochemistry
6. Flow cytometry: Positive markers: CD38, CD138, CD56, monoclonal cytoplasmic Ig; negative markers: surface Igs, B-cell markers (CD19, CD20, CD22), CD45 dim
7. Ig gene rearrangement
8. Common cytogenetic abnormalities: 14q32 translocation with 11q13, 6p21, 4p16, 16q23, or 20q11; 13/13q14 loss; trisomies of chromosome 3, 5, 7, 11, 15, 19, or 21

mutations of N-ras and K-ras, which are present mainly in medullary relapse (1). The up-regulation of bcl-2 expression may be due to ras activation, loss of p53 function, or both (33).

Ig gene rearrangement has been found in most cases of MM studied, and its detection rate is proportional to the degree of plasma cell infiltration in the bone marrow (37). When myeloma cells are <10%, no Ig gene is rearranged. Myeloma cells have a high frequency of Ig V_H gene somatic mutation, which is consistent with derivation from a post-germinal center, antigen-driven B cell (3). The salient features for laboratory diagnosis of MM are summarized in Table 6.24.2.

Clinical Manifestations

As mentioned before, the 2008 WHO classification includes two asymptomatic subtypes of MM, which are the smoldering MM and MGUS (6). The indolent subtype in the old WHO classification is deleted, so that it is consistent with the International Myeloma Working Group classification (5).

In the Mayo Clinic studies, approximately one quarter of the MGUS patients developed MM, macroglobulinemia, amyloidosis (Fig. 6.24.18), or related lymphoproliferative disorders, with an actuarial rate of 16% at 10 years, 33% at 20 years, and 40% at 25 years (5). The risk of MGUS to develop MM or related disorders was about 1% per year. The most important risk factor is high concentration of serum paraprotein. However, patients with Bence–Jones proteinuria, even in a high concentration, may remain in a stable condition for many years (5).

As mentioned before, the International Myeloma Working Group emphasizes end organ damage as the major diagnostic criterion for symptomatic myeloma. The major

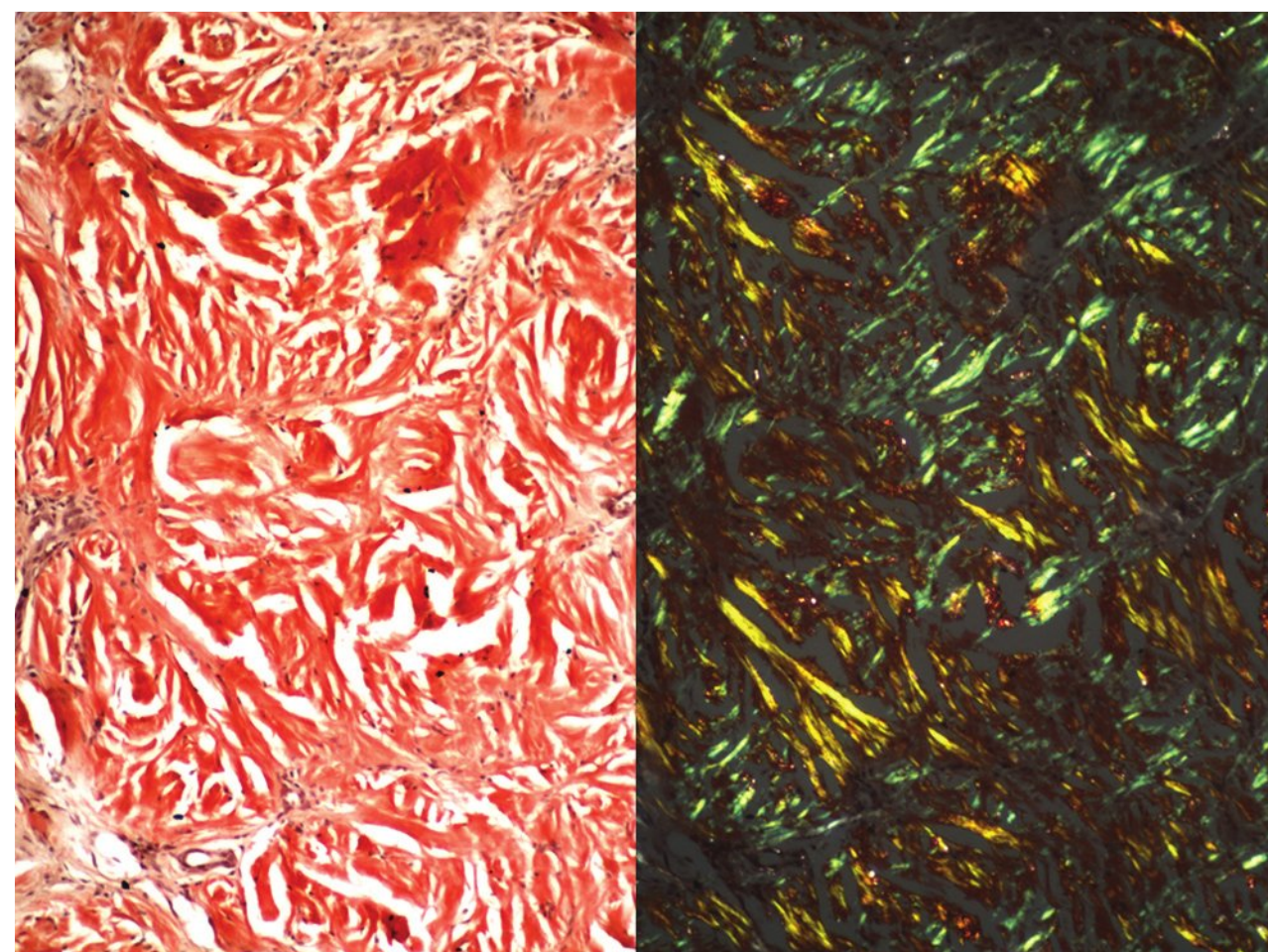


FIGURE 6.24.18 Tongue biopsy from a patient with macroglossia shows amyloidosis demonstrated by Congo red stain (**left**) and birefringence (**right**). 10 × magnification.

clinical manifestations in end organ damage are increased serum calcium, renal insufficiency, anemia, and lytic bone lesions, a syndrome referred to as CRAB (5). Recurrent infections are also considered to be an integral part of the clinical symptoms. This concept is now fully incorporated in the 2008 WHO classification.

There are three major myeloma staging systems—namely, Durie–Salmon, Medical Research Council, and Merlini–Waldenström–Jayakar. The Durie–Salmon system is the most popular and has been adopted by WHO with minor modification. It is based on the hemoglobin level, serum calcium value, bone x-ray film, and paraprotein quantity to divide myeloma into three stages (Table 6.24.3) (6,38). The stages are further subdivided into A and B depending on whether the renal function is normal or abnormal. This staging system has a clear relationship to patient survival.

As mentioned before, MM patients may develop PCL, which has a frequency between 2% and 4% of all MM cases. PCL may present de novo in patients with no previous evidence of MM (primary form) or as a leukemic phase in the terminal stage of MM (secondary form) (11). About 60% of PCL cases are primary, whereas 40% are secondary. The primary form is seen in younger patients, with a higher incidence of hepatosplenomegaly and lymphadenopathy, a higher platelet count, fewer lytic bone lesions, lower serum M-protein levels, and longer survival than the secondary form (3,25). The response to chemotherapy in PCL is poor, and the median survival of these patients is about 1 to 2 months. (11).

Most myeloma is of the IgG class (58.6% to 60.3%), whereas the frequencies of plasma cell dyscrasia involving IgA and IgM are similar (about 15%) (39). When IgM is involved, the condition is traditionally called Waldenström macroglobulinemia, because IgM is a macromolecule and, in this entity, osteolytic bone lesion is usually not present. The incidence of light-chain disease ranges from 5% to 15% in different series (39). The incidence of IgD myeloma is

TABLE 6.24.3

WHO Modified Durie and Salmon Staging System for MM

Stage I:

- Low M-protein levels: IgG < 50 g/L, IgA < 30 g/L, Urine BJ < 4 g/24 h
- Absent or solitary bone lesions
- Normal hemoglobin, serum calcium, Ig levels (non-M-protein)

Stage II:

- Overall values between I and III

Stage III:

- Any one or more of the following
- High M-protein: IgG > 70 g/L, IgA > 50 g/L, Urine light chain > 12 g/24 h
- Advanced, multiple lytic bone lesions
- Hemoglobin < 8.5 g/dL, Serum calcium > 12 mg/dL

Subclassification: Based on renal function

- A = serum creatinine < 2 mg/dL
- B = serum creatinine $\geq$ 2 mg/dL

about 1%, whereas that of IgE myeloma and heavy-chain diseases is <1% (39).

When two monoclonal components are identified, the condition is called biclonal gammopathy (40). Although its reported frequency is about 2.4% to 4.1%, the real incidence is probably even lower, because the two components in some cases may represent heavy- or, less frequently, light-chain switching, occurring in the same clone of myeloma cells (40,41). An easy way to distinguish biclonal from monoclonal myeloma is to use the double-staining immunofluorescence technique to detect the cytoplasmic Igs with two fluorochromes (Figs. 6.24.19 and 6.24.20) (39). If two monoclonal components are found in the same cells, the conclusion should be monoclonal gammopathy with

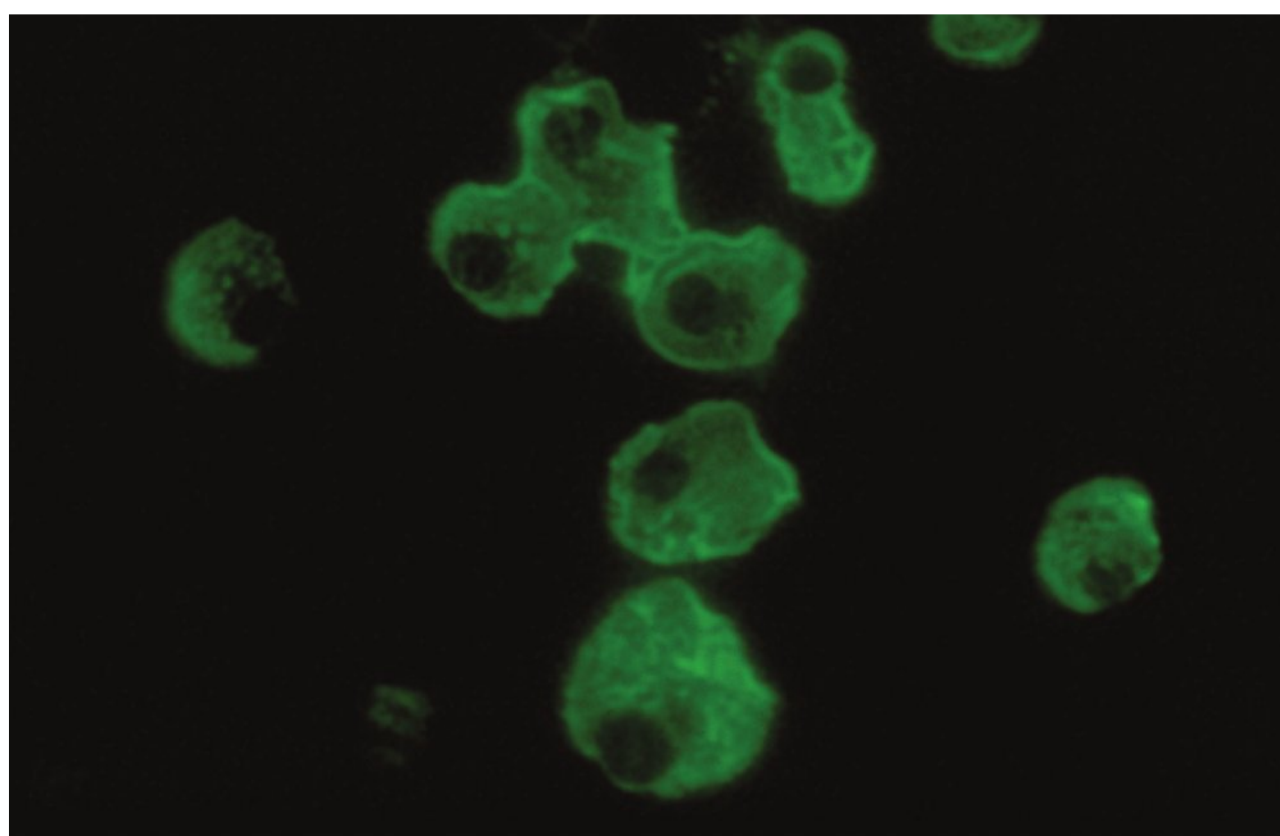


FIGURE 6.24.19 Fluorescein-conjugated anti-IgG antibody stains positive in the cytoplasm of myeloma cells. Immunofluorescence stain, 100 $\times$ magnification.

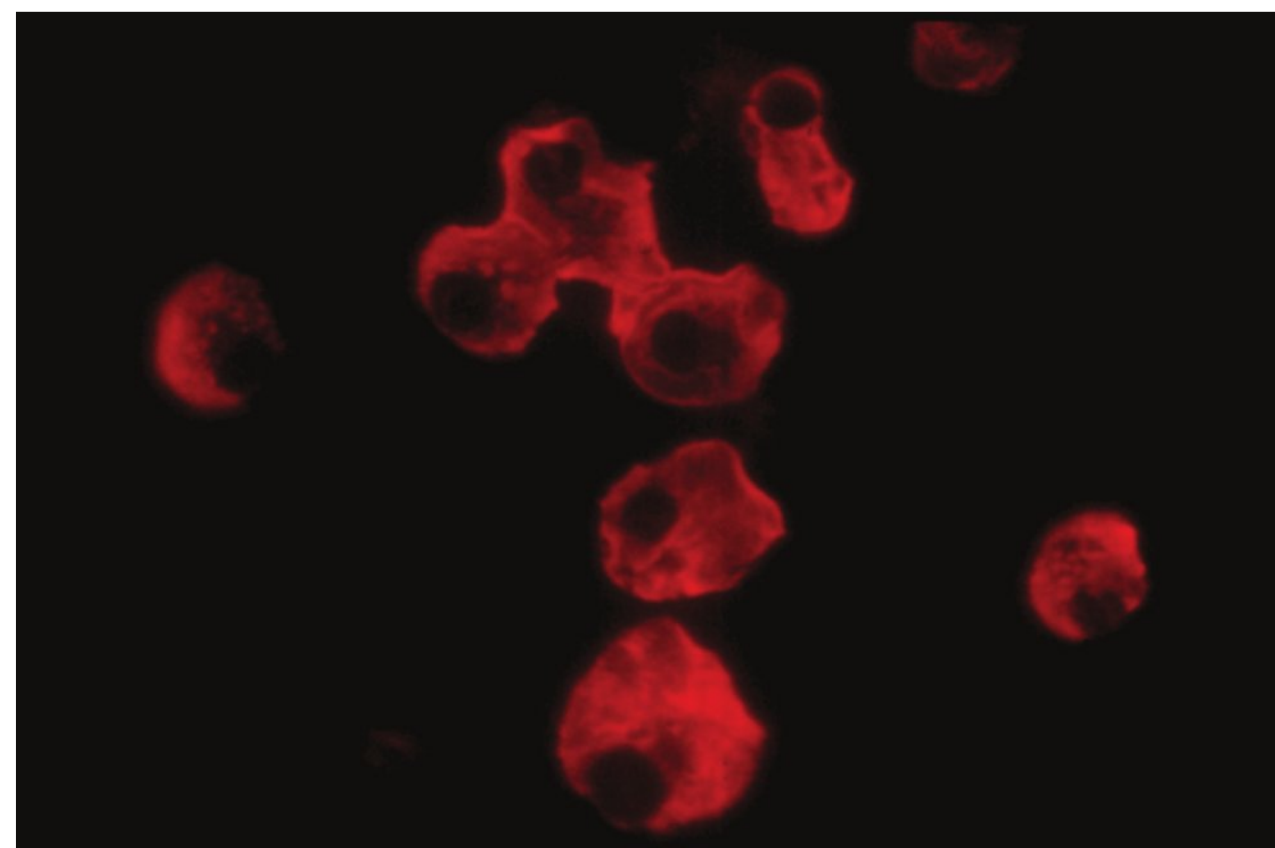


FIGURE 6.24.20 Rhodamine-conjugated anti-IgA antibody stains positive in the cytoplasm of the same group of myeloma cells as demonstrated in Figure 6.22.15, indicating that both IgG and IgA are produced by the same clone of myeloma cells. Immunofluorescence stain, 100 $\times$ magnification.

heavy-chain switching. Among cases of biclonal gammopathy, 65% are MGUS, 19% lymphoproliferative disorders, and 16% MM (42). The clinical features, therapeutic response, and survival of patients with biclonal MM do not differ from those with monoclonal MM (42).

Since 2001, sFLC assay is available commercially for the diagnosis and therapeutic monitoring of MM. These are nephelometric/turbidimetric assays using latex-conjugated polyclonal antibodies against the light-chain epitopes that are hidden when light chains are bound to heavy chains, but exposed when light chains circulate freely. The sFLC assay is considered abnormal when the clonal free light chain is increased above normal ranges and the kappa/lambda ratio is abnormal. This assay has proven to be very useful for the diagnosis of MM, particularly oligosecretory MM and light-chain disease, as well as AL amyloidosis (12,13,15,43). It is also helpful in therapeutic monitoring and prediction of prognosis. The sFLC assay has been fully integrated into the International Uniform Response Criteria in MM and in AL amyloidosis (Table 6.24.4).

Many parameters can be used to predict the prognosis in MM cases (2). As advocated by the Mayo Clinic, the most sensitive technique is plasma cell labeling index (PCLI). The mononuclear cells isolated from the patient's bone marrow are incubated with 5-bromo-2-deoxyuridine (BUD-URD), and the uptake of BUD-URD is detected by fluorochrome-labeled monoclonal antibody BU-1. BUD-URD is incorporated into the nucleus of cells synthesizing DNA; therefore, cells showing BU-1 antibody are those in the S phase. A PCLI of $\geq$ 1% is classified as high.

The S phase can be directly measured by flow cytometry. Although this technique measures more cells that synthesize DNA than does PCLI, it is time consuming and inaccurate when plasma cells in the patient's bone marrow are <20% (42).

Two helpful tests routinely done in clinical laboratories are serum B₂M and LDH levels. The levels of B₂M reflect both tumor mass and renal function (27). A high level of B₂M represents a large tumor mass and poor renal function, thus

TABLE 6.24.4

International Myeloma Working Group Uniform Response Criteria for MM

Complete response (CR)	Negative immunofixation of serum and urine ≤5% plasma cells in bone marrow or disappearance of plasmacytoma
Stringent CR	CR as defined above Normal sFLC ratio Absence of clonal cells in bone marrow by immunohistochemistry or immunofluorescence
Very good partial response	Serum and urine M-protein detectable by immunofixation but not on electrophoresis; or ≥90% reduction in serum M-protein plus urine M-protein <100 mg/24 h
Partial response	≥50% reduction of serum M-protein and reduction in 24-h urinary M-protein by ≥90% or to <200 mg/24 h, or 50% or more decrease in the difference between clonal and non-clonal sFLC levels
Progressive disease	Increase of ≥25% from baseline in any one or more of the following: serum M-protein (absolute ≥ 0.5 g/dL), urine M-protein (absolute ≥ 200 mg/24 h), difference between clonal and nonclonal sFLC levels (absolute >10 mg)
Stable disease	Not meeting criteria for the above conditions

sFLC, serum free light chain.

identifying a high-risk group (44). A high level of LDH, in contrast, defines a high-grade myeloma with a rapidly progressive clinical course (27). Therefore, patients with high LDH levels usually have shorter remission and shorter survival.

As mentioned before, cytogenetic abnormalities often carry a poor prognosis, especially those involving chromosome 11 or 13 and translocations. One study found that the median PCL was 1.5% in patients with cytogenetic aberrations and 0.2% in patients with normal cytogenetics (2). Plasmablastic morphology and circulating plasma cells are also predictors of poor prognosis. Patients with MM show a short survival when cutaneous metastasis occurs (Fig. 6.24.21) (45).

In the terminal stage, MM may transform into lymphoma, acute lymphoblastic leukemia, or acute myelomonocytic leukemia, or it may become drug resistant and actively proliferative. These phenomena are called phenotypic escapes (17).

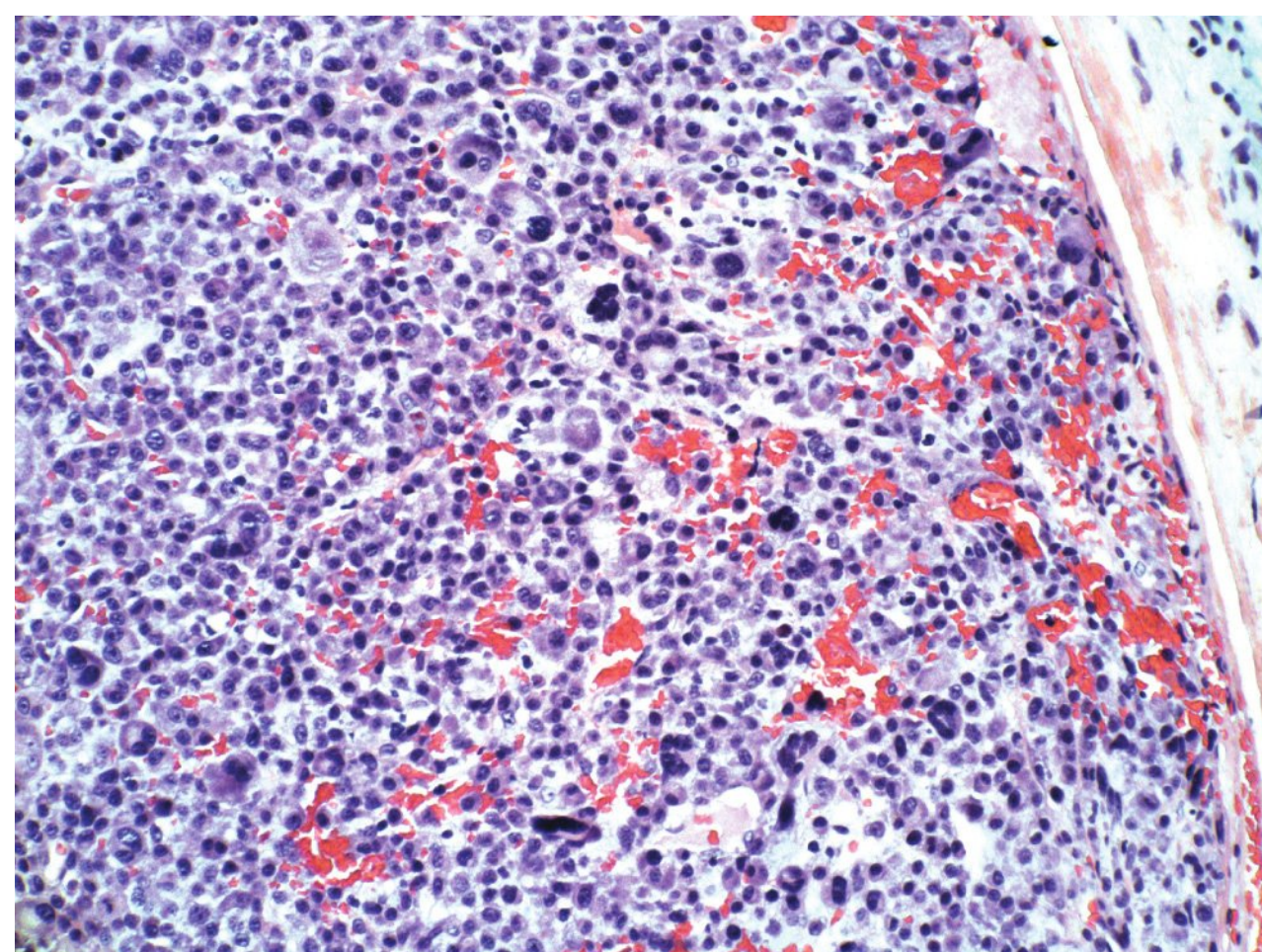


FIGURE 6.24.21 Skin biopsy shows extensive pleomorphic myeloma cell infiltration with many multinucleated giant cells. Hematoxylin and eosin, 20 × magnification.

There are two discoveries that may have potential implications in the pathogenesis of and therapeutic approach to MM (1,46,47). The first one is that IL-6 mediates the autocrine and paracrine growth of MM cells. The bone marrow stromal cells in MM patients secrete large amounts of IL-6; this secretion has a paracrine effect on tumor growth and prevents apoptosis (47). Using murine anti-IL-6 monoclonal antibodies for the treatment of advanced MM has produced some effect but no lasting benefit (48). The second discovery is Kaposi sarcoma herpesvirus or human herpesvirus-8 (HHV-8) in the bone marrow dendritic cells of patients with MM. The bone marrow microenvironment with HHV-8-infected dendritic cells has a growth and anti-apoptosis advantage over uninfected stromal cells (47). Thus, HHV-8 may play an important role in the pathogenesis of MM. If this association is confirmed, novel therapeutic strategies targeting this virus would be beneficial to MM patients.

Traditionally, MM is treated with melphalan, vincristine, doxorubicin, and dexamethasone in various combinations. Recently, the use of thalidomide and bortezomib has further improved therapeutic effects (49). Two therapeutic approaches are now being explored simultaneously: The first one is to aim at complete cure, and the second one is to convert MM into a chronic indolent disease. The current therapeutic protocols are summarized in a review article by Kyle and Rajkumar (50).

REFERENCES

1. Bataille R, Harousseau JL. Multiple myeloma. *N Engl J Med*. 1997;336:1657–1664.
2. Rajkumar SV, Greipp PR. Prognostic factors in multiple myeloma. *Hematol Oncol Clin North Am*. 1999;13:1295–1314.
3. Grogan TM, Van Camp B, Kyle RA, et al. Plasma cell neoplasms. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:142–156.

4. Hideshima T, Bergsagel PL, Kuehl WM, et al. Advances in biology of multiple myeloma: clinical applications. *Blood*. 2004;104:607–618.
5. The International Myeloma Working Group. Criteria for the classification of monoclonal gammopathies, multiple myeloma and related disorders: a report of the International Myeloma Working Group. *Br J Haematol*. 2003;121:749–757.
6. McKenna RW, Kyle RA, Kuehl WM, et al. Plasma cell neoplasms. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:200–213.
7. Brunning RD, McKenna RW. *Tumor of the Bone Marrow*. Washington, DC: Armed Forces Institute of Pathology; 1994:323–367.
8. Bartl J, Frisch B, Fasteh-Moghdam A, et al. Histologic classification and staging of multiple myeloma. A retrospective and prospective study of 574 cases. *Am J Clin Pathol*. 1987;87:342–355.
9. Shaheen SP, Tahwalkar SS, Medeiros LJ. Multiple myeloma and immunosecretory Disorders. *Adv Anat Pathol*. 2008;15:196–210.
10. Heerema-McKenney A, Waldron J, Hughes S, et al. Clinical, immunophenotypic, and genetic characterization of small lymphocyte-like plasma cell myeloma. A potential mimic of mature B-cell lymphoma. *Am J Clin Pathol*. 2010;133:265–270.
11. Blade J, Kyle RA. Nonsecretory myeloma, immunoglobulin D myeloma, and plasma cell leukemia. *Hematol Oncol Clin North Am*. 1999;13:1259–1272.
12. Pratt G. The evolving use of serum free light chain assays in haematology. *Br J Haematol*. 2008;141:413–422.
13. Dispenzieri A, Kyle R, Merlini G, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*. 2009;23:215–224.
14. Dimopoulos MA, Kiamouris C, Mouloupoulos LA. Solitary plasmacytoma of bone and extramedullary plasmacytoma. *Hematol Oncol Clin North Am*. 1999;13:1249–1257.
15. Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. *Leukemia*. 2009;23:3–9.
16. Thakhi A, Edinger M, Myles J, et al. Flow cytometric immunophenotyping of non-Hodgkin's lymphomas and related disorders. *Cytometry*. 1996;25:113–124.
17. Grogan TM, Spier CM. B-cell immunoproliferative disorders, including multiple myeloma and amyloidosis. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1557–1587.
18. Witzig T, Kimlinger T, Ahmann G, et al. Detection of myeloma cells in the peripheral blood by flow cytometry. *Cytometry*. 1996;26:113–120.
19. Van Camp B, Dutie BGM, Spier C, et al. Plasma cells in multiple myeloma express a natural killer cell-associated antigen. CD56 (NKH-1): Leu 19. *Blood*. 1990;76:377–382.
20. Rawstron AC, Davies FE, DasGupta R, et al. Flow cytometric disease monitoring in multiple myeloma: the relationship between normal and neoplastic plasma cells predicts outcome after transplantation. *Blood*. 2002;100:3095–3100.
21. Harada H, Kawano MM, Huang N, et al. Phenotypic difference of normal plasma cells from mature myeloma cells. *Blood*. 1993;81:2658–2663.
22. Lin P, Owens R, Tricot G, et al. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. *Am J Clin Pathol*. 2004;121:482–488.
23. O'Connell FR, Pinkus JL, Pinkus GS. CD138 (Syndecan-1), a plasma cell marker: immunohistochemical profile in hematopoietic and nonhematopoietic neoplasms. *Am J Clin Pathol*. 2004;121:254–263.
24. Boyer-Garmer IB, Sandreson RD, Dhodapkar MV, et al. Syndecan-1 (CD138) immunoreactivity in bone marrow biopsies of multiple myeloma: shed syndecan-1 accumulates in fibrotic regions. *Mod Pathol*. 2001;14:1052–1058.
25. Garcia-Sanz R, Orfao A, Gonzalez M, et al. Primary plasma cell leukemia: clinical, immunophenotypic, DNA ploidy, and cytogenetic characteristics. *Blood*. 1999;93:1032–1037.
26. Ward MS. The use of flow cytometry in the diagnosis and monitoring of malignant hematological disorders. *Pathology*. 1999;31:382–392.
27. Wei A, Juneja S. Bone marrow immunohistology of plasma cell neoplasms. *J Clin Pathol*. 2003;56:460–411.
28. Barlogie B, Epstein J, Selvanayagam P, et al. Plasma cell myeloma—new biological insights and advances in therapy. *Blood*. 1989;73:865–879.
29. Drach J, Gatringer C, Huber H. Multiple myeloma with co-expression of myeloid and natural killer cell antigens. *Blood*. 1990;76:265–267.
30. Epstein J, Xiao H, He XY. Markers of multiple hematopoietic-cell lineages in multiple myeloma. *N Engl J Med*. 1990;322:664–668.
31. Omede P, Boddadoro M, Gallone G, et al. Multiple myeloma. Increased circulating lymphocytes carrying plasma cell-associated antigens as an indicator of poor survival. *Blood*. 1990;76:1345–1379.
32. Fonseca R, Coignet LJA, Dewald GW. Cytogenetic abnormalities in multiple myeloma. *Hematol Oncol Clin North Am*. 1999;13:1169–1180.
33. Feinman R, Sawyer J, Hardin J, et al. Cytogenetics and molecular genetic in multiple myeloma. *Hematol Oncol Clin North Am*. 1997;11:1–25.
34. Chesi M, Bergsagel P, Brents L, et al. Dysregulation of cyclin D1 by translocation into an IgH gamma switch region in two multiple myeloma cell lines. *Blood*. 1996;88:674–681.
35. Drach J, Ackermann J, Fritz E, et al. Presence of a p53 gene deletion in patients with multiple myeloma predicts for short survival after conventional-dose chemotherapy. *Blood*. 1998;92:802–809.
36. Liebisch P, Dohner H. Cytogenetics and molecular cytogenetics in multiple myeloma. *Eur J Cancer*. 2006;42:1520–1529.
37. Humphries JE, Dressman HK, Williams ME. Immunoglobulin gene rearrangement in multiple myeloma. Limitation of Southern blot analysis. *Hum Pathol*. 1991;22:966–971.
38. Durie BGM. Staging and kinetics of multiple myeloma. *Semin Oncol*. 1986;13:300–309.
39. Sun T. *Interpretation of Protein and Isoenzyme Patterns in Body Fluids*. New York: Igaku-Shoin; 1991:33–60.
40. Kyle RA, Robinson RA, Katzmman JA. The clinical aspects of biclonal gammopathies. Review of 57 cases. *Am J Med*. 1981;71:999–1088.
41. Saltman DL, Banks JAR, Ross FM, et al. Molecular evidence for a single clonal origin in biphenotypic concomitant chronic lymphocytic leukemia and multiple myeloma. *Blood*. 1989;74:2062–2065.
42. Kyle RA, Rajkumar SV. Monoclonal gammopathies of undetermined significance. *Hematol Oncol Clin North Am*. 1999;13:1181–1202.
43. Durie BG, Harousseau JL, Miguel JS, et al. International uniform response criteria for multiple myeloma. *Leukemia*. 2006;20:1467–1473.

44. Greipp PR, Katzmann JA, O'Fallon WM, et al. Value of b₂-microglobulin level and plasma cell labeling indexes as prognostic factors in patients with newly diagnosed myeloma. *Blood*. 1989;74:2062–2065.
45. Requena L, Kutsner H, Palmedo G, et al. Cutaneous involvement in multiple myeloma. *Arch Dermatol*. 2003;139:475–486.
46. Anderson K. Advances in the biology of multiple myeloma. Therapeutic applications. *Semin Oncol*. 1999;26:10–22.
47. Berenson JR. Etiology of multiple myeloma. What's new? *Semin Oncol*. 1999;26:2–9.
48. Bataille R, Barlogie B, Lu ZY, et al. Biologic effects of anti-interleukin-6 murine monoclonal antibody in advanced multiple myeloma. *Blood*. 1995;86:3043–3049.
49. Kyle RA, Rajkumar SV. Multiple myeloma. *N Engl J Med*. 2004;351:1860–1873.
50. Kyle RA, Rajkumar SV. Multiple myeloma. *Blood*. 2008;111:2962–2972.

CASE 25

Hairy Cell Leukemia

CASE HISTORY

A 52-year-old man presented with fatigue, decreased exercise tolerance, and dyspnea on exertion for 6 months. The patient was in good health 6 months before presenting, and his only medical problem was alcoholism. Upon consultation with his private care physician, he was found to have pancytopenia and was admitted for further evaluation.

Physical examination on admission showed a healthy-looking man with no apparent distress. Abdominal examination found a large spleen, but liver was not palpable. There was no peripheral lymphadenopathy.

Hematology workup revealed a total leukocyte count of 1,200/mL with 38.5% lymphocytes, 56.6% neutrophils, 2.6% monocytes, 1.6% eosinophils, and 0.5% basophils. His hematocrit was 33.3%, hemoglobin 11.4 g/dL, and platelets 72,000/mL.

After admission, a bone marrow biopsy was performed and showed 90% cellularity with 70% being “hairy cell.” Myeloid cells were markedly decreased, but megakaryocytes and erythroid series were relatively normal.

He was treated with a cycle of cladribine and subsequently developed neutropenic fever. His condition was finally under control with antibiotics and granulocyte-colony-stimulating factor. His leukocyte count returned to normal in 2 months. A follow-up examination of peripheral blood and bone marrow showed no leukemic cells 1 year later.

FLOW CYTOMETRIC FINDINGS

Blood: CD5 23%, CD19 77%, CD19/CD5 9%, CD20 80%, CD22 85%, CD11c 95%, CD22/CD11c 85%, CD23 5%, CD25 90%, CD103 97%, FMC-7 92%, CD19/k 63%, CD19/l 6%, CD10 77%, CD14 2%, CD45 100% (Fig. 6.25.1).

Bone marrow: CD5 11%, CD19 91%, CD19/CD5 7%, CD22 95%, CD11c 99%, CD22/CD11c 95%, CD23 6%, CD25 91%, CD103 95%, FMC-7 93%, CD19/k 67%, CD19/l 7%, CD10 90%, CD14 2%, CD45 100%.

IMMUNOCHEMISTRY

Bone marrow biopsy: The tumor cells were positive for CD20, DBA-44, and tartrate-resistant acid phosphatase (TRAP).

DISCUSSION

Hairy cell leukemia (HCL) is a rare disease with an incidence of 2% of lymphoid leukemias (1,2). However, it is an important entity in the differential diagnosis of the low-grade lymphoproliferative disorders. Recent advances in the treatment of HCL enable a long-term remission in most patients; thus, an accurate diagnosis of this leukemia has become more meaningful than ever before.

Morphology

Typical hairy cells are usually of medium size (10 to 15 nm) with a moderate amount of loose-textured cytoplasm (Fig. 6.25.2) (3). The cytoplasmic projections are delicate (hairy) and cover the entire cell surface, compared with the villi on the villous lymphocytes of splenic lymphoma, which are polar in distribution (4). The homogeneous, ground-glass chromatin pattern in the nucleus of hairy cells is also in contrast to the clumped chromatin patterns in tumor cells from chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL), and splenic lymphoma with villous lymphocytes (currently, splenic B-cell marginal zone lymphoma [SMZL]) (5). Electron microscopy may help to distinguish the villi on hairy cells from those of villous lymphocytes of SMZL and to identify the ribosome-lamella complex in hairy cells (Fig. 6.25.3).

In contrast to other leukemias, the histologic patterns in various organs are very specific for HCL (Table 6.25.1). In the bone marrow, hairy cells are packed “back to back” and show an oval or kidney-shaped nucleus with abundant clear unstained cytoplasm that separates the nuclei widely, and give a pattern variably described as “fried egg,” “sponge,” or “honeycomb” (Fig. 6.25.4) (3,6,7).

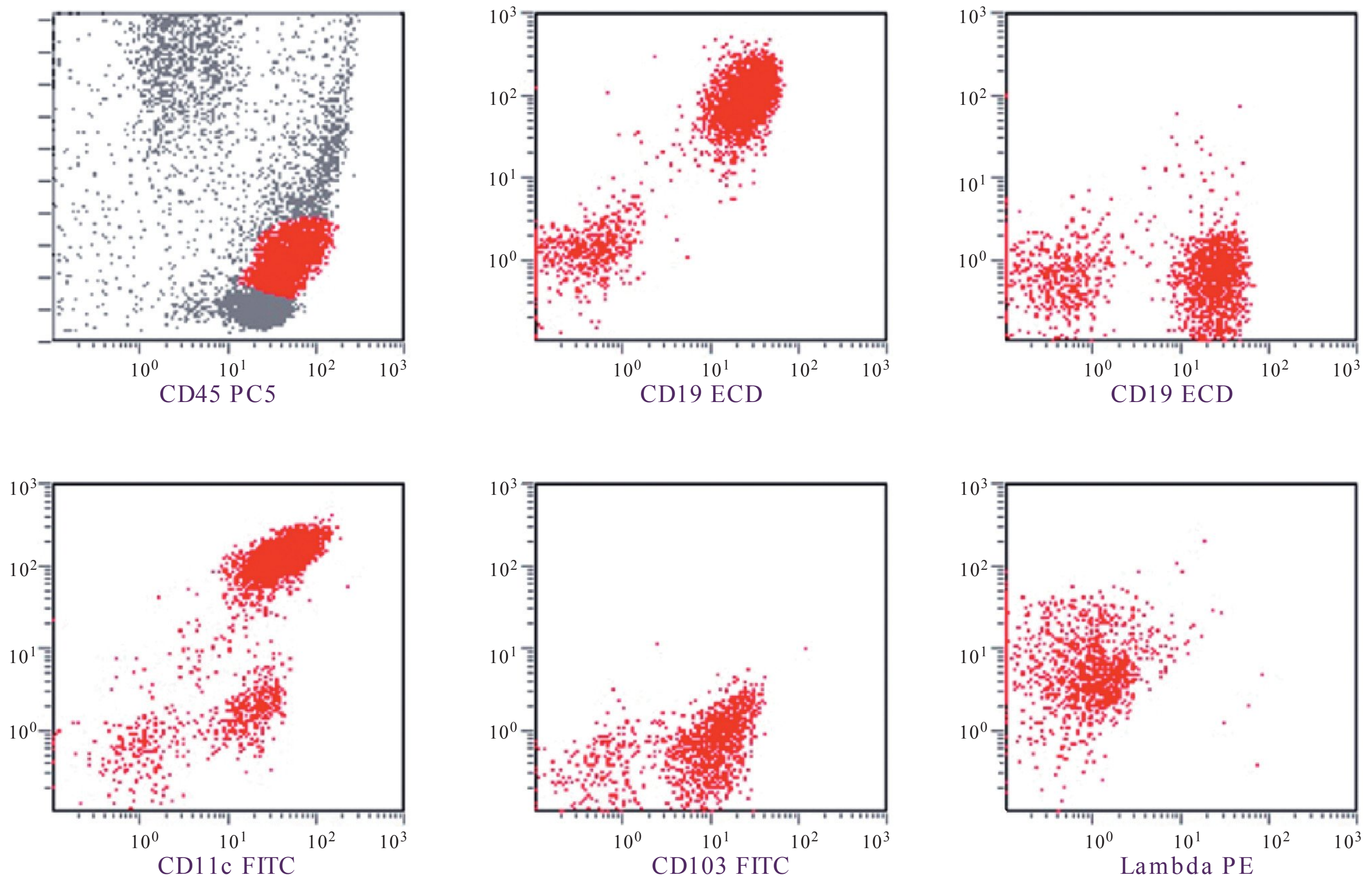


FIGURE 6.25.1 Flow cytometric histograms show CD19, FMC-7, CD11c, CD22, and CD103 reactions in a monoclonal k B-cell population. CD23 is negative. SS, side scatter; PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ECD, phycoerythrin–Texas Red; RD1, rhodamine.

Because the tumor cells are closely packed, the cell borders appear to be interlocking. A reticulin stain may demonstrate increased reticulin fibers in the area infiltrated by tumor cells, but collagen fibrosis is typically absent in HCL. Reticulin fibrosis in HCL is partly due to its production of

fibronectin (2,8). An intrasinusoidal infiltration pattern can also be seen in up to 73% of HCL cases, but lymphoid nodules are typically not found in HCL, and this characteristic can be used to distinguish it from SMZL (9).

In the spleen, HCL is characterized by involving solely the red pulp, both the cords and the sinuses. Probably because of the destruction of the splenic cords and atrophy of the white pulp, variably dilated sinuses are filled with erythrocytes and form the so-called blood lakes. When these lakes are lined by hairy cells, they are called pseudosinuses (Fig. 6.25.5) (3,7,10). In full-blown HCL, the red pulp cords are totally destroyed and the entire spleen is composed of the tumor cells intermixing with pools of erythrocytes. Although this pattern has been occasionally encountered in other conditions, such as CLL, chronic myeloid leukemia, and multiple myeloma (11), in an appropriate clinicopathologic setting, this pattern is diagnostic of HCL.

In the liver, both portal areas and sinuses are involved by the tumor cells. Leukemic infiltration of the sinusoid wall may produce angiomatous lesions (10,12).

Peripheral lymphadenopathy is observed in only 5% to 10% of patients, but abdominal and mediastinal adenopathies are frequently found at autopsy (7). The rarity of lymph node involvement is probably due to the fact that HCL cells lack the L-selectin for interaction with high endothelial venules at the hilum of lymph nodes and the absence of chemokine

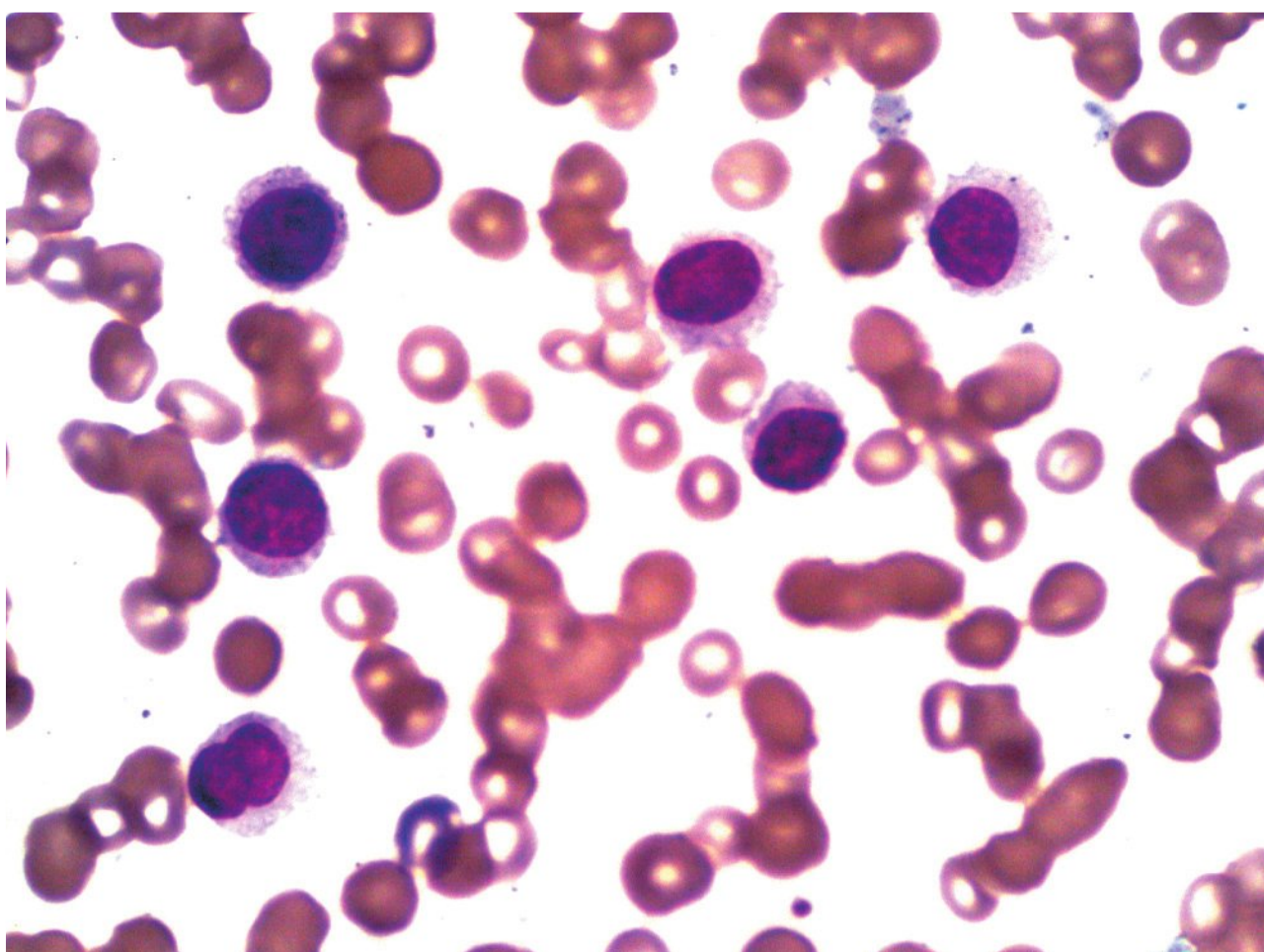


FIGURE 6.25.2 Peripheral blood smear shows six leukemic hairy cells with moderate cytoplasm and multiple cytoplasmic projections. A binucleated cell is present. Wright–Giemsa, 100× magnification.

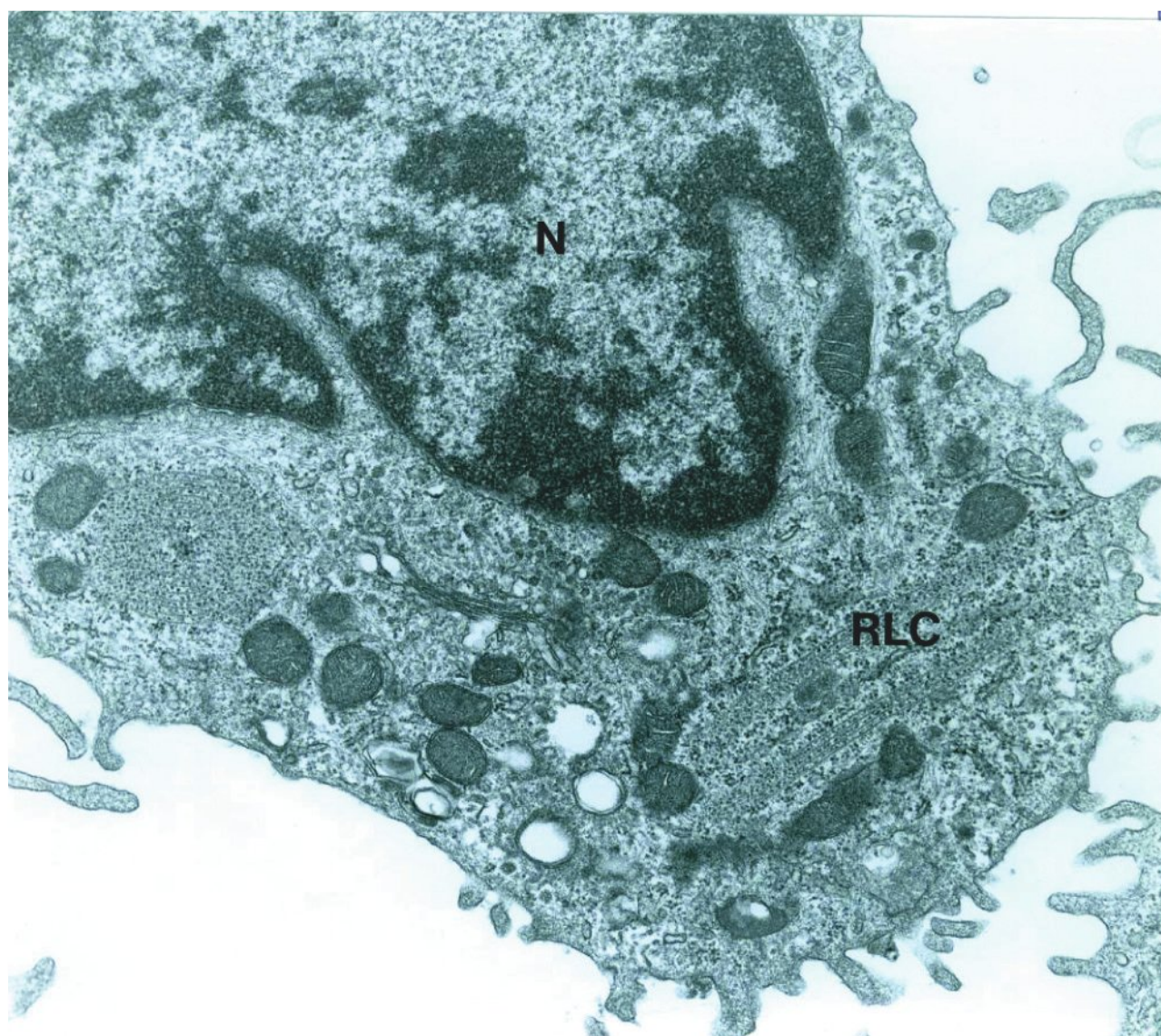


FIGURE 6.25.3 Electron micrograph shows a hairy cell with many cytoplasmic projections and two characteristic ribosome–lamellar complexes (RLC). N, nucleus. 27,500× magnification. (Courtesy of Dr. Saul Teichberg, North Shore University Hospital, New York.)

receptor CCR7 for transendothelial migration (2,8). When peripheral lymphadenopathy is found, high-grade lymphoma transformation should be suspected (13). The normal architecture of lymph nodes is partially or completely replaced by leukemic cells, which often surround the lymph follicles (Fig. 6.25.6). A fried egg pattern is also frequently seen.

Tumor cells of HCL may also infiltrate skin, lungs, kidneys, stomach, intestine, myocardium, meninges, adrenals,

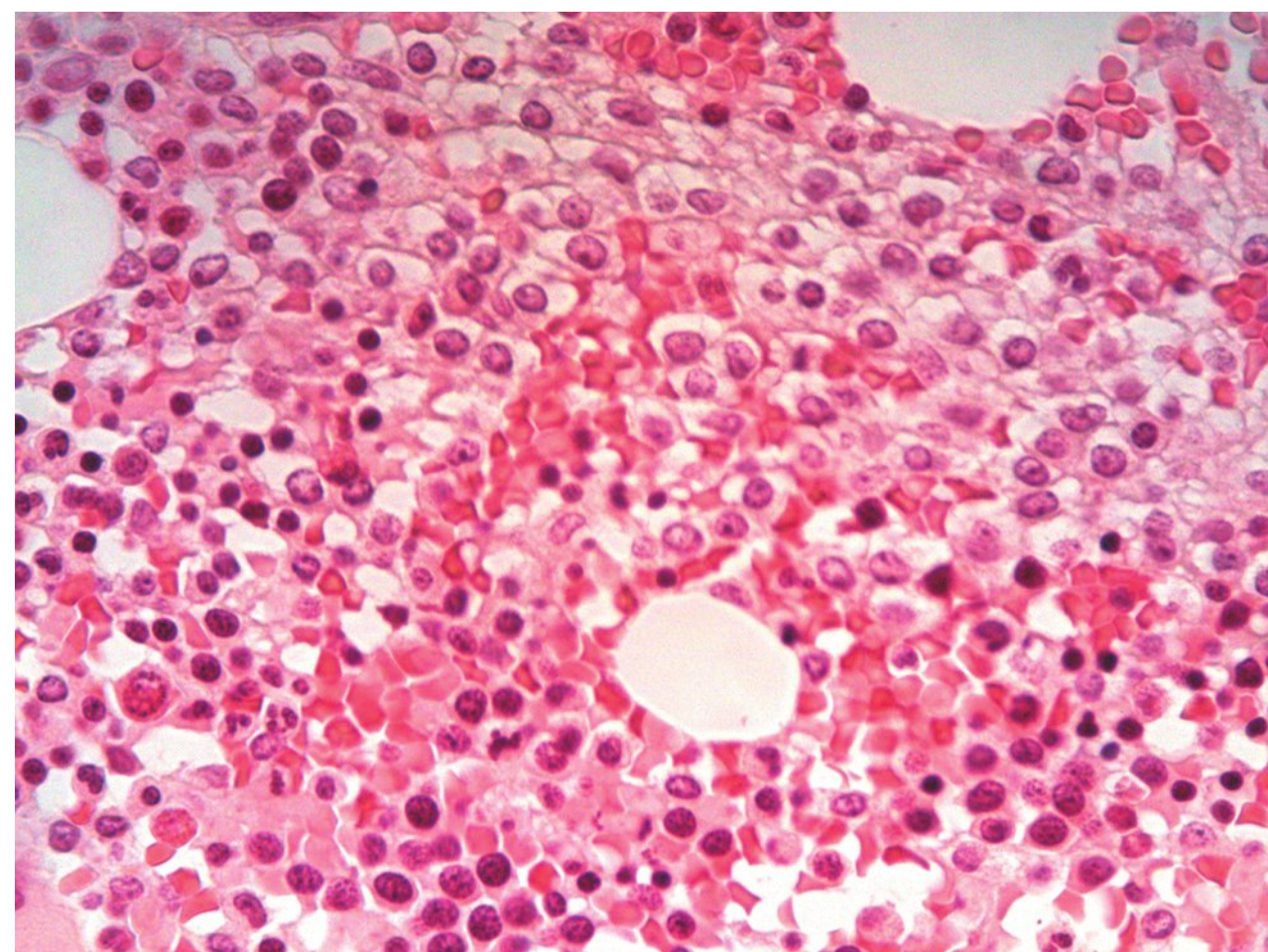


FIGURE 6.25.4 Bone marrow biopsy shows closely packed hairy cells with clear cytoplasm and prominent cell border (honeycomb pattern). Hematoxylin and eosin, 60× magnification.

and pancreas, but the histologic pattern is not specific and the infiltrates seldom cause clinical symptoms (14).

The recent improvement of the histologic techniques further facilitates the diagnosis of HCL in tissue sections. Examples include the demonstration of ribosome–lamella complexes in plastic-embedded sections (15) and the visualization of hairy projections on tumor cells in tissue sections stained with monoclonal antibody DBA-44 (Fig. 6.25.7) (16). Immunohistochemical stains for DBA-44 and CD20 are particularly helpful in detecting small numbers of tumor cells in early cases of HCL.

Immunophenotype

Because clinical and laboratory features may overlap among various low-grade B-cell lymphoproliferative

TABLE 6.25.1

Characteristic Morphologic Features of HCL

Histologic patterns	Bone marrow: fried egg pattern Spleen: blood lake or pseudosinus formation Liver: angiomatous lesion Lymph node: fried egg pattern surrounding or replacing follicles
Cytology	Medium-sized, moderate cytoplasm with cytoplasmic projections, homogeneous ground-glass chromatin pattern of nucleus
Special features	Special cytologic features in blood and bone marrow aspirate, and typical histologic patterns in tissues

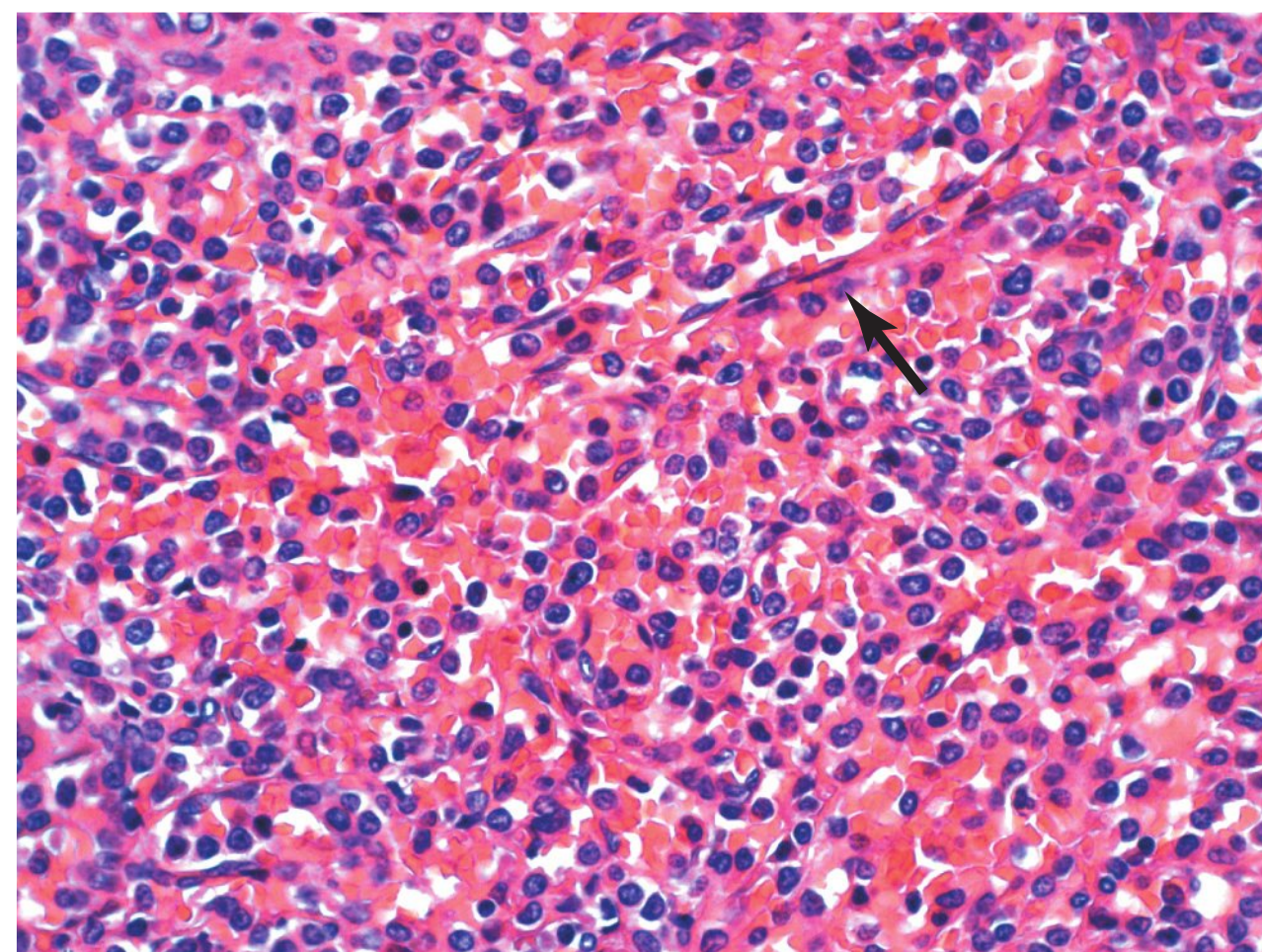


FIGURE 6.25.5 Splenectomy specimen shows pseudosinuses surrounded by leukemic cells, as compared with one residual sinus in this field (black arrow). Hematoxylin and eosin, 40× magnification.

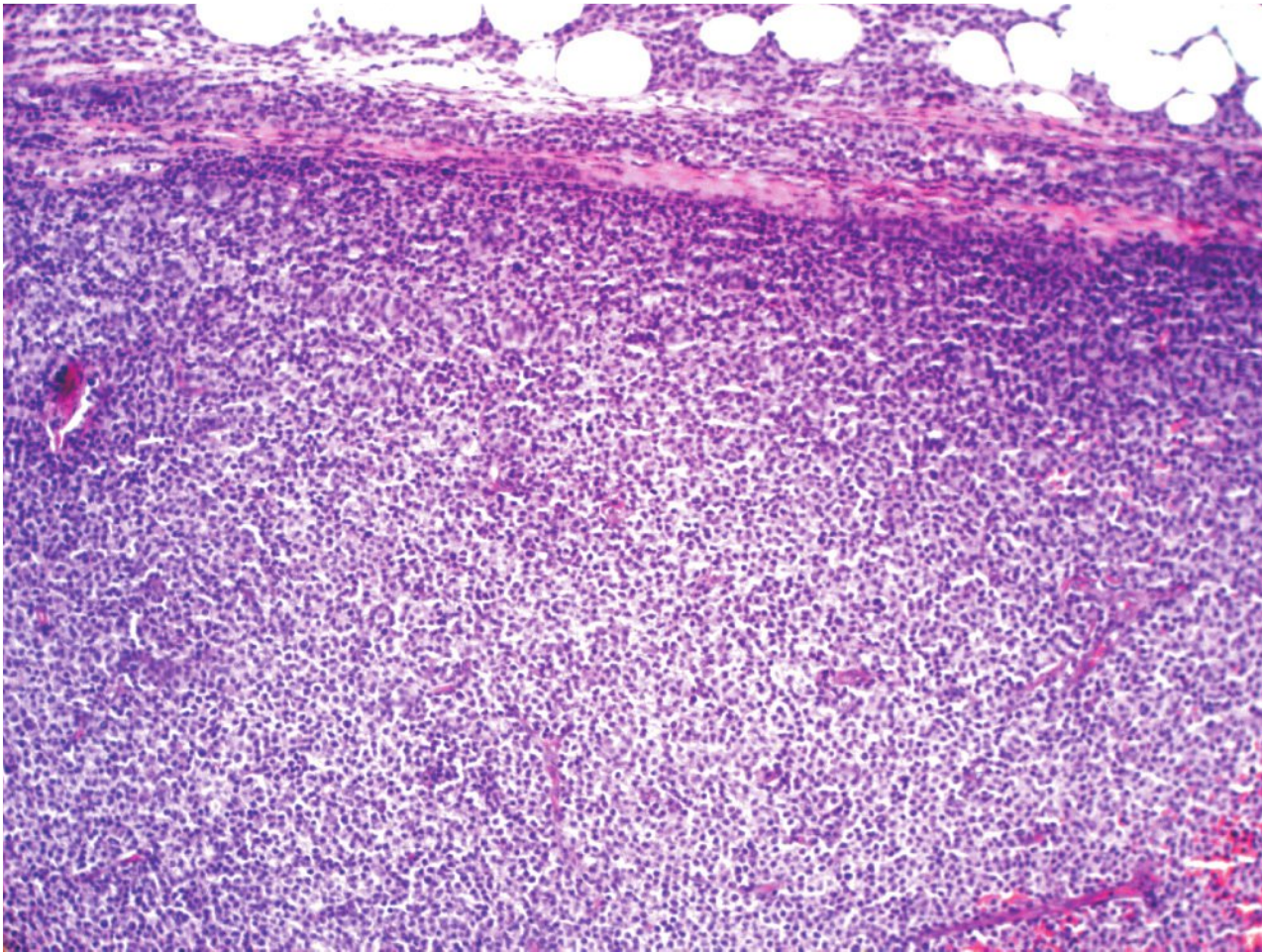


FIGURE 6.25.6 Lymph node biopsy shows total effacement of normal architecture by diffuse leukemic cell infiltrate. Note the wide space between cells representing the clear cytoplasm of leukemic hairy cells. Hematoxylin and eosin, 10× magnification.

disorders, immunophenotyping is particularly useful for differential diagnosis. All B-cell tumors share a monoclonal surface immunoglobulin (Ig) pattern (IgM in most cases, but IgG in HCL variants [HCLvs]). One unique feature of HCL is the expression of preswitched (IgM/IgD) and post-switched (IgG/IgA) Igs by the same cells, as demonstrated by flow cytometry (9) and by RNA transcript analysis of single cells (17,18). In one study, the frequency was as high as 40% (17). Unlike other B-cell tumors, the coexpression of multiple isotypes in HCL is not due to the existence of a heterogeneous population, and only a single light-chain type is present.

B-cell neoplasms also share some common pan-B antigens, such as CD19, CD20, and CD79a, but CD22 was first identified in HCL and is not shared by all B-cell tumors. The

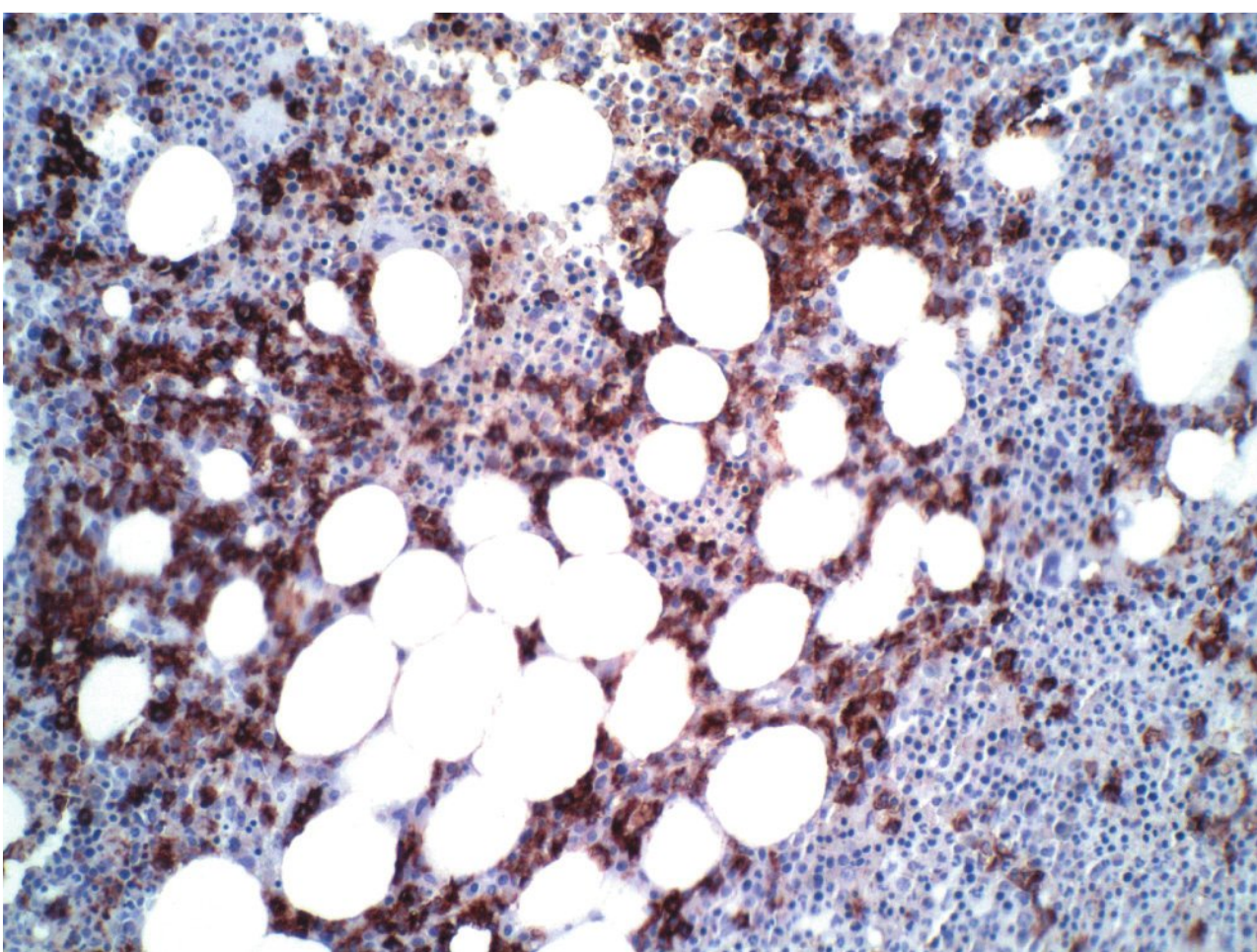


FIGURE 6.25.7 Bone marrow biopsy shows DBA-44 staining of the leukemic cells. Immunoperoxidase, 20× magnification.

Ig-associated antigen, CD79b, in contrast, is characteristically absent in HCL (1,7). The coexpression of a B-cell antigen with CD11c, a monocyte antigen, is another unusual feature of HCL. The finding of dual CD22 and CD11c staining on HCL cells was initially considered highly specific for HCL (19,20). However, this coexpression has been found in >10% of lymphocytes in a variety of B-cell proliferative disorders, including CLL, PLL, SMZL, and nodal marginal zone lymphomas (19,21,22). Therefore, one should look for the subtle differences between HCL and other lymphoid neoplasms.

First, HCL usually shows strong fluorescence for both CD22 and CD11c and is well defined in flow cytometric contourgrams, but the staining of CD22 and CD11c in other lymphoid tumors is weaker and less well defined than HCL (19). Second, other lymphoid tumors show a much higher percentage of CD22+ CD11c- population than HCL cases; most cells are CD11c positive in the latter (17). Another study found that the coexpression of CD20 and CD11c was even more specific than CD22/CD11c for HCL, because this phenotype was not found in other cases of lymphomas as well as acute and chronic leukemias studied (23).

Another important marker for the identification of HCL is CD25 (Tac antigen or interleukin [IL]-2R) (24). CD25 is present in human T-cell lymphotropic virus type I (HTLV-I)-associated adult T-cell leukemia/lymphoma (25), and occasionally in Hodgkin lymphoma (26) and diffuse large B-cell lymphoma (27). However, it is very helpful in distinguishing HCL from other low-grade B-cell neoplasms, which are usually negative for CD25. The only exception is SMZL, which showed positive CD25 reaction in 25% of cases in one study (28). The same study revealed that CD103 and HCL-associated antigen 2 (HC2) were most helpful in distinguishing HCL from SMZL; both markers were generally not positive in SMZL.

In addition, elevated levels of the soluble form of IL-2 receptor (IL-2R; CD25) are present in the sera of HCL patients (29). The IL-2R level correlates well with the tumor cell concentration in the bone marrow and the patient's clinical status.

FMC-7 is usually included in the HCL study panel, but it is not as specific as the above-mentioned markers; it is also positive for PLL and most cases of nodal marginal zone B-cell lymphoma and mantle cell lymphoma (1,30). Bcl-2 antigen is usually positive, but no bcl-2 gene rearrangement is identified in HCL cases (7). Recently, two new antibodies have been used, with promising results, to diagnose HCL. The first one is annexin A1 (ANXA1), which is the product of the ANXA1 gene that is up-regulated in HCL cases by gene-profiling analysis (31). Immunohistochemical staining with this antibody was found to be highly specific and sensitive for differential diagnosis of B-cell lymphomas. However, ANXA1 is also expressed by T cells and myeloid cells and should be used side by side with CD20 staining (2). The second one is CD123, which identifies the α chain of the human IL-3R. Flow cytometry showed that it was positive in 95% of typical HCL cases, but negative in 91% cases of HCLv and in 97% cases of SMZL (32). T-bet transcription factor has also been reported to be helpful in the detection of minimal residual disease for HCL (33).

Other frequently positive but not specific antigens for HCL include HLA-DR and plasma cell-associated antigen-1 (PCA-1), which may also help in differential diagnosis (3,23). PC-1 and some early appearing B-cell antigens, such as CD21 and CD24, are usually negative (34,35).

The negative reactions to CD5, CD10, and CD23 are useful in excluding small lymphocytic lymphoma/CLL, mantle cell lymphoma, and follicular lymphoma. However, a recent study showed that CD10 and CD23 can be present in a small percentage of HCL cases (33). In other reports, CD10 has been found in 5% to 26% and CD23 in 20% of HCL cases (36). On the other hand, some important markers, such as CD103 and CD25, can be absent in a few cases of HCL (36). The demonstration of bcl-1 (cyclin D1) in 37% of HCL cases is alarming and may cause confusion with the diagnosis of mantle cell lymphoma (37).

Recent studies have indicated that the presence of CD103 and CD11c, absence of CD25, and infrequent presence of TRAP are highly characteristic of HCLs, which should be distinguished from HCL because of their marked differences in therapeutic response and prognosis (9).

TRAP is a time-honored marker for the diagnosis of HCL. Although many studies claimed to detect acid phosphatase-positive stains in other lymphoid neoplasms, most of these reports did not adhere to the strict definition of real TRAP positivity. In HCL, the acid phosphatase staining is heavy (>40 granules) and diffuse (not focal), and the staining intensity before and after tartrate treatment should be comparable (not markedly different) (Fig. 6.25.8). By summarizing their 15-year experience, Yam et al. (38) found that false-positive or false-negative TRAP results were due to either technical or interpretive errors. Subsentive techniques and incorrect criteria for result interpretation were the major problems. After eliminating these problems, TRAP is, indeed, highly sensitive and specific. However, in exceptional conditions, positive results can be seen in other lymphoid tumors and negative results in HCL.

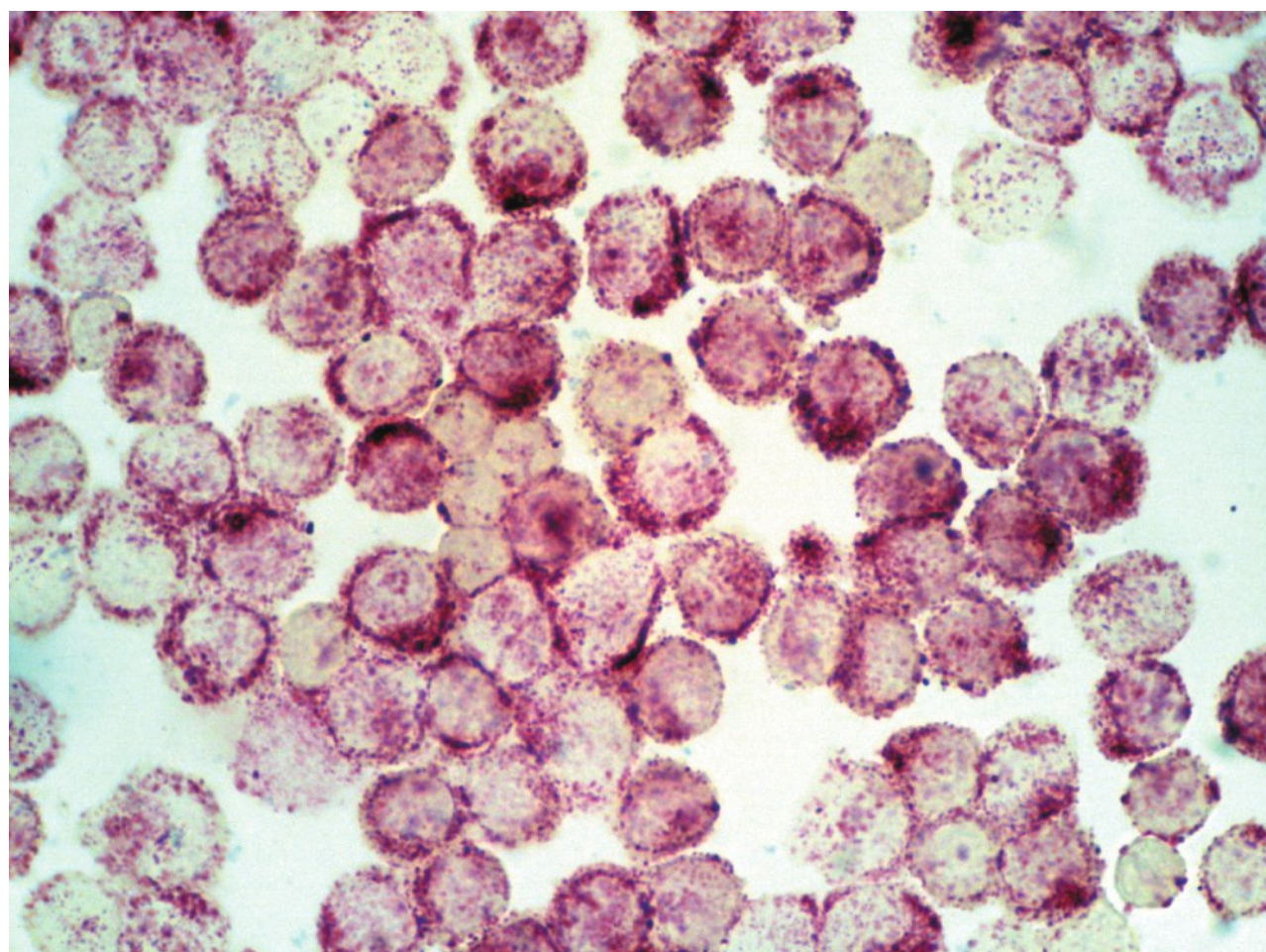


FIGURE 6.25.8 Tartrate-resistant acid phosphatase staining of the leukemic cells on a cytopsin of bone marrow aspirate. Note the high percentage and diffuse, heavy staining of the cells. Cytochemical stain, 100× magnification.

Recent studies have indicated that the presence of CD103, CD11c, and DBA-44, but absence of CD25, ANXA1, CD123, HC2, and infrequent presence of TRAP are highly characteristic of HCLv, which should be distinguished from HCL because of its marked differences in therapeutic response and prognosis (9,39,40). A study of 215 cases of CD103-positive B-lymphoproliferative disorders showed that HCL cases were consistently positive for CD25 (37). CD103+CD25– cases, on the other hand, showed morphologic features of HCLv, SMZL, and occasional prolymphocytic leukemia and diffuse large B-cell lymphoma (37). This group of cases was also characterized by the absence of ANXA1 expression, peripheral leukocytosis, and poor response to purine analogs, such as cladribine and pentostatin (37). The markers that help to distinguish HCL from similar B-cell lymphomas are summarized in Table 6.25.2 (2,51).

Two cases of HCL of T-cell lineage have been reported (41,42). Both cases were believed to be associated with infections by retrovirus human T-cell lymphotropic virus type II (HTLV-II). Reexamination of one of the two cases revealed that the case was actually composed of B-cell HCL and CD8-positive T-cell leukemia (43). The HTLV-II genome was found in the DNA of the leukemic T cells but not in the leukemic hairy cells (44). Severe T-cell dysfunction has been found in HCL cases (45).

Comparison between Flow Cytometry and Immunohistochemistry

The peripheral blood in HCL cases is characteristically pancytopenic, so flow cytometry may not be able to detect the hairy cells. The bone marrow aspirate may be difficult to obtain when reticulin fibrosis is present. Therefore, a definitive diagnosis sometimes depends on immunohistochemical staining. DBA-44 and CD20 are frequently used to identify hairy cells in bone marrow; however, CD20 is nonspecific for HCL, and DBA-44 can also be present in other lymphomas, particularly follicular lymphoma (46). The combination of DBA-44 and TRAP (Fig. 6.25.9) stains in tissue sections is considered most sensitive and specific for the diagnosis of HCL. In one study, this combination is positive in all 86 cases of HCL and none of 66 cases of other lymphomas (47). Another study demonstrates this combination pattern in all 56 cases of HCL but only in 3% of cases of other non-Hodgkin lymphomas (46). The combined use of ANXA1 and CD20 is also a good alternative (2). However, if bone marrow aspirate is available or the blood specimen contains many tumor cells, flow cytometry is most reliable for the diagnosis by using a panel of CD11c/CD22, CD25, CD103, and FMC-7. One important note for gating of HCL is that the HCL cell cluster is usually located in the monocyte rather than the lymphocyte gate (37).

Molecular Genetics

HCL cases usually show Ig gene but not T-cell receptor gene rearrangements (24). Therefore, despite the frequent demonstration of myelomonocytic markers and of phagocytic activities in hairy cells (23), HCL is still considered a B-cell neoplasm (19). Gene rearrangement does not help in differential diagnosis between HCL and other B-cell neoplasms. However, one study found that HCL may have more than

TABLE 6.25.2

Immunophenotypic Differences between HCL, HCLv, SRPL, and SMZL

Markers	HCL	HCLv	SRPL	SMZL
CD20	Bright	Bright	Bright	Bright
CD22	Bright	Bright	Bright	Moderate
CD11c	100% bright	87% bright	97% moderate	33% faint
CD76	Bright	Bright	86%	45%
CD103	100% bright	60% faint to moderate	38% variable	Negative
CD123	95% bright	9%	16% faint	Negative
CD25	Bright	Dim to negative	Faint	Variable
CD27	Negative	Negative	Negative	Normal
ANXA1	Positive	Negative	Negative	Negative
HC2	Positive	Negative	N/A	Negative
TRAP	Positive	Dim to negative	Negative	Weak to negative

ANXA1, annexin A1; HCL, hairy cell leukemia; HCLv, hairy cell leukemia variant; N/A, data not available; SMZL, splenic B-cell marginal zone lymphoma; SRPL, splenic diffuse red pulp small B-cell lymphoma; TRAP, tartrate-resistant acid phosphatase.

one neoplastic clone; one was sensitive to chemotherapy, but another one was refractory (48). Therefore, genotyping may be occasionally useful for the evaluation of therapeutic problems.

Analysis of the Ig heavy-chain variable (IGHV) gene in one study showed somatic mutation in most cases of HCL (18). In addition, HCL cases showed multiple isotype expression. Therefore, Forconi et al. (18) hypothesize that HCL cells are arrested at the point where multiple isotypes can be generated but before deletional switch takes place.

Recent studies have demonstrated that somatic mutation is useful to distinguish HCL from HCLv and correlate well with the prognosis (49–51). Based on the IGHV mutational studies, HCLv shows more similarities with SMZL

than with HCL (51). HCL cases with unmutated IGHV genes are refractory to single-agent cladribine and have more aggressive behavior (52). Another powerful prognostic predictor is the usage of VH4-34 gene. Cases expressing VH4-34 gene are frequently associated with unmutated IGHV gene, high leukocyte count, lower response rate and progression-free survival after initial cladribine, and shorter overall survival from diagnosis (50). This gene is more frequently demonstrated in HCLv than HCL cases, but the prognosis is the same in either entity with VH4-34 expression (50).

A study with comparative expressed sequence hybridization showed a homogeneous gene expression profile in all HCL cases (53). HCL cases also expressed a spleen signature, which was not found in the normal lymph node. As cells that are present in the spleen and not lymph nodes include only sinusoidal lining cells (endothelial cells) and marginal zone B cells, this finding suggested the marginal zone origin of HCL (53).

A study of gene expression profiling by Basso et al. (54) drew a similar conclusion in that HCL cases displayed a homogeneous pattern and were of memory B cell origin. It is known that the marginal zone is composed of a heterogeneous population including memory B cells. HCL cells belong to a small subset of marginal zone B cells that lacks CD27 expression. This finding explains the possible reason why chromosomal translocation is not found in HCL cases. The mechanism that can generate these aberrations is switched off in memory cells (54).

The study by Basso et al. (54) also identified several genes that could explain the clinical behavior of HCL. For instance, the down-regulation of CCR7 gene, a chemokine receptor the deficiency of which hampers the ability of B cells to enter lymph nodes, may explain why HCL cells seldom spread to lymph nodes (54). The up-regulation of ANXA1 explains why HCL cells have the capacity of phagocytosis, as

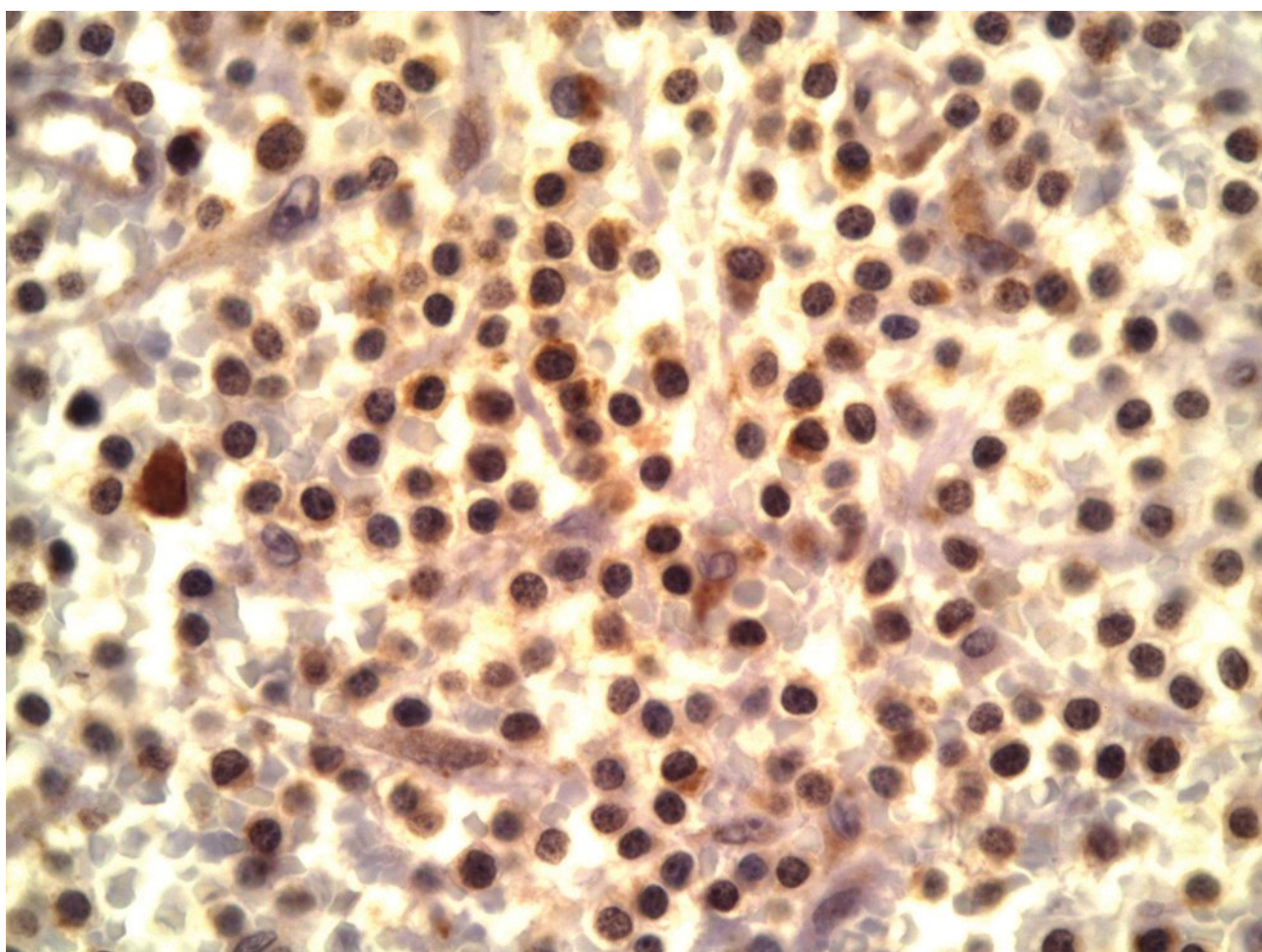


FIGURE 6.25.9 HCL involving the spleen shows positive TRAP staining. Note that the leukemic cells replace the sinusoidal lining cells. Immunoperoxidase, 100× magnification.

ANXA1 is required for phagocytic uptake of *Brucella suis* by human monocytes (54). The overexpression of IL-3Ra and Fms-like tyrosine kinase 3 may explain bone marrow fibrosis in HCL cases, because the ligands for these two receptors are important for adhesion of B cells to fibronectin (54).

Cytogenetic studies may demonstrate various anomalies in HCL, including clonal aberration, numeric changes, and structural alterations in various chromosomes (3,55,56). However, except for one claim of frequent finding of 5q13 (32), no consistent cytogenetic abnormality has been established in HCL (1,3).

Recently, parathyroid adenoma 1 (PRAD1)/CCND1 has been found to be overexpressed in most cases of HCL; in about one third of these cases, its level of expression approaches that seen in mantle cell lymphoma (6). However, this expression is not associated with t(11;14), and the cyclin D1 protein was detected only by Western blotting and not by immunohistochemical staining in earlier studies (7). Recent studies, nevertheless, are able to demonstrate cyclin D1 by immunohistochemistry (8,37).

The current patient is a typical case of HCL. The clinical presentation is pancytopenia with splenomegaly but not lymphadenopathy. Immunophenotypically, positive CD19, CD20, CD11c/CD22, CD25, CD103, and FMC-7 were demonstrated by flow cytometry; positive CD20, DBA-44, and TRAP were detected by immunohistochemical stains. The only atypical finding is the positive reaction to CD10, which can be seen in some HCL cases as previously reported. A honeycomb histologic pattern was shown in the bone marrow biopsy. The patient promptly responded to cladribine therapy with complete remission in subsequent years.

The salient features for laboratory diagnosis of HCL are summarized in Table 6.25.3.

Clinical Manifestations

Clinically, most patients with HCL have splenomegaly (Fig. 6.25.10); however, 10% to 20% of cases show no

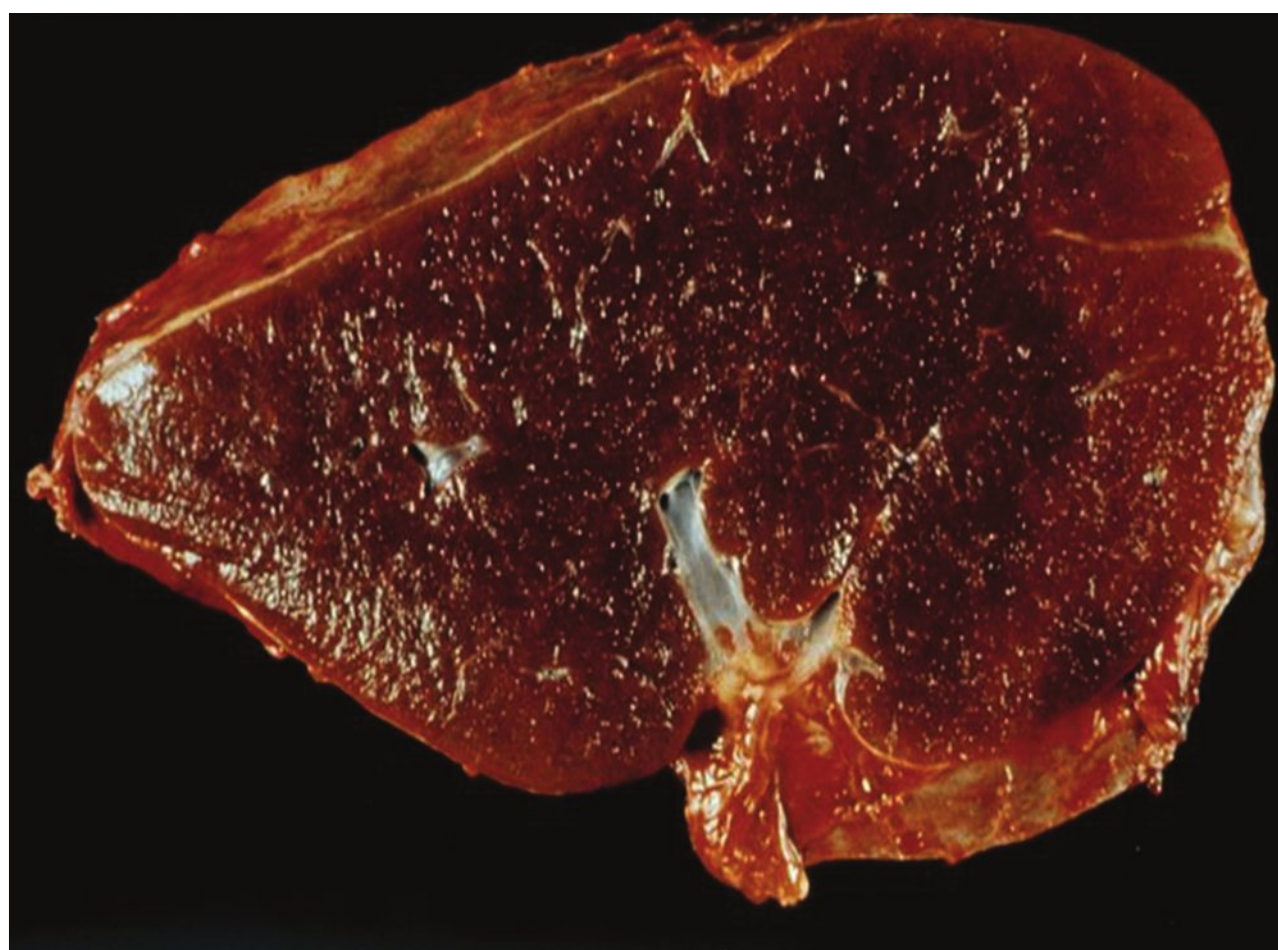


FIGURE 6.25.10 Large spleen in a case of HCL shows dark-red cut surface representing many coalesced “blood lakes.” Note that there are no prominent white nodules as seen in other lymphomas.

TABLE 6.25.3

Salient Features for Laboratory Diagnosis of HCL

1. Hairy projections on leukemic cells
2. Pancytopenia with monocytopenia
3. Tartrate-resistant acid phosphatase positivity on smears or tissue sections
4. Characteristic immunophenotype:
Positive for CD11c/CD22, CD25, FMC-7, CD103, CD123, and HC2
Special immunohistochemical marker: DBA-44 and ANXA1
5. Monoclonal surface immunoglobulin pattern
6. Electron microscopy: cytoplasmic projections and ribosome–lamella complex
7. Immunoglobulin gene rearrangement

TRAP, tartrate-resistant acid phosphatase.

palpable spleen (34). About one third of patients have hepatomegaly, but lymphadenopathy is an unusual feature in HCL and occurs mainly in HCLv (3). Skin, bone, and the central nervous system are seldom involved. The clinical course is usually chronic and indolent: Patients may have a stable condition and do not require treatment until severe pancytopenia occurs. Nevertheless, rare complications may occur, including paravertebral mass, meningitis, mediastinal mass with superior vena cava syndrome, massive abdominal lymph nodes with ascites, gastrointestinal involvement with protein-losing enteropathy, rupture of spleen, and opportunistic infections (34). For unknown reasons, atypical mycobacterial infection, particularly *Mycobacterium Kansaii* infection, is relatively common in HCL patients (Fig. 6.25.11) (57). On rare occasions, HCL

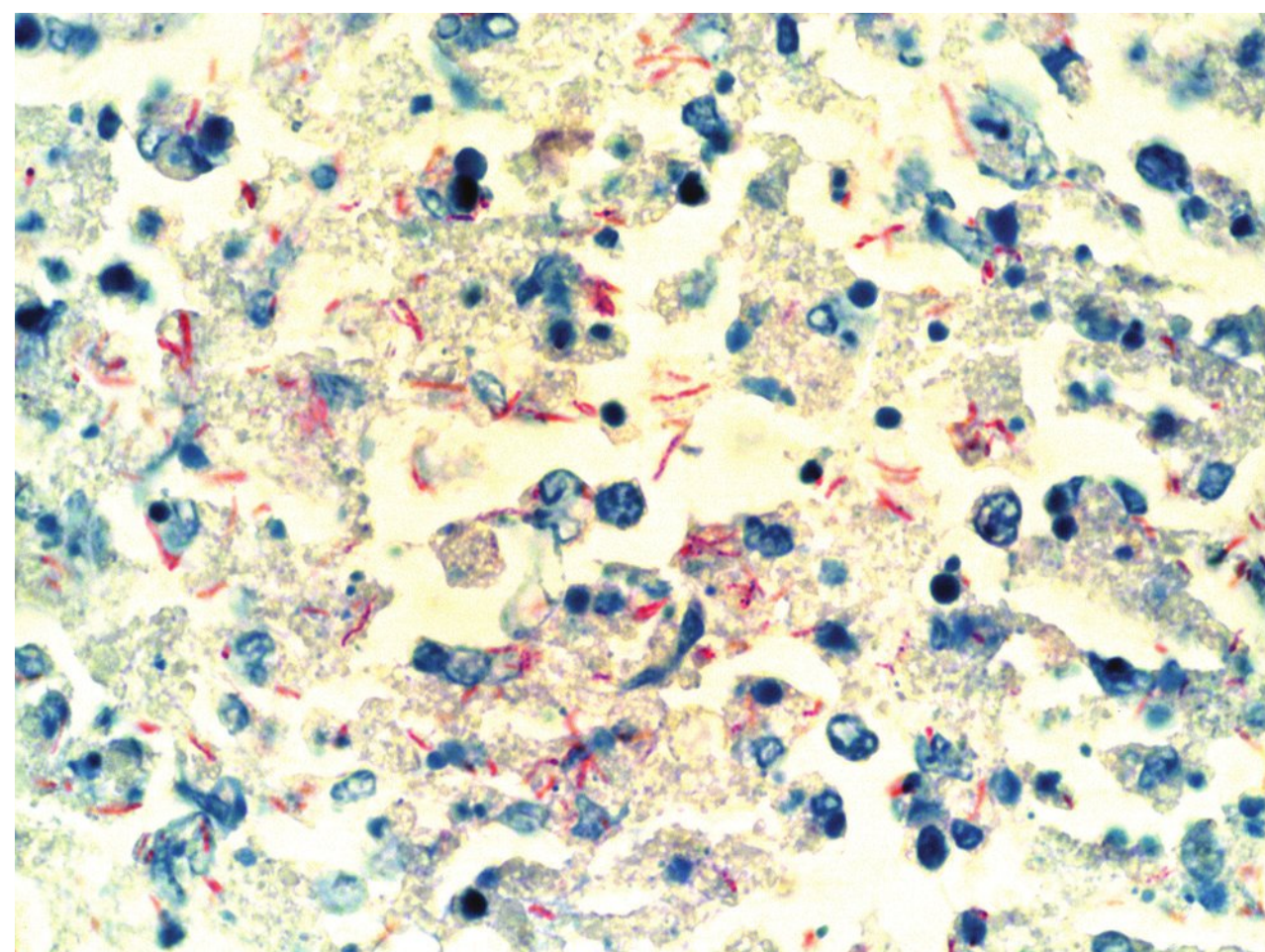


FIGURE 6.25.11 Spleen section in a case of HCL shows numerous acid-fast bacilli, identified as *M. kansasii* by culture. AFB stain, 100× magnification.

may transform into a high-grade lymphoma that is probably associated with p53 mutation (13).

The major laboratory feature is pancytopenia with hemoglobin levels between 7 and 10 g/dL and platelet counts <80,000/mL (3). The pancytopenic feature, particularly monocytopenia, is useful to distinguish HCL from other leukemia/lymphoma, such as SMZL. If a monocyte count is normal or increased, a diagnosis of HCL should be questioned, unless it is an HCLv. Cytopenia is probably the result of splenic sequestration of blood cells as well as inhibition of hematopoiesis in the bone marrow (7).

A dry tap on marrow aspirate is also a characteristic result of reticulin fibrosis in the bone marrow. The morphology of hairy cells in bone marrow aspirate is frequently not typical (Fig. 6.25.12). Therefore, a bone marrow biopsy should be done routinely, and it is frequently diagnostic.

Cases of HCLv have been reported with increasing frequency (1,9,40). In contrast to HCL, the variant usually shows high leukocyte count with no monocytopenia, tumor cells with prominent nucleoli, absence of reticulin fibrosis in the bone marrow, and a different immunophenotype, as mentioned before. It is important to recognize the HCLv because it does not respond to conventional HCL treatments (such as interferon α , cladribine, and pentostatin) and its prognosis is worse than that of HCL. HCLv is also designated as type II HCL, or hybrid form of HCL. Thus far, the hybrid forms are mainly composed of HCL/PLL (58) or HCL/CLL (22,59,60).

Melo et al. (4) suggested that HCL, HCLv, SMZL, and PLL represent a spectrum of cell types frozen at slightly different stages during late B-cell maturation. This statement has been supported by several in vitro experiments that show induction of CLL and PLL into HCL by tetradecanoyl phorbol acetate (61,62). Therefore, hybrid features are not uncommonly seen in these closely related low-grade B-cell neoplasms, and clear-cut diagnosis may not be always achievable in every case.

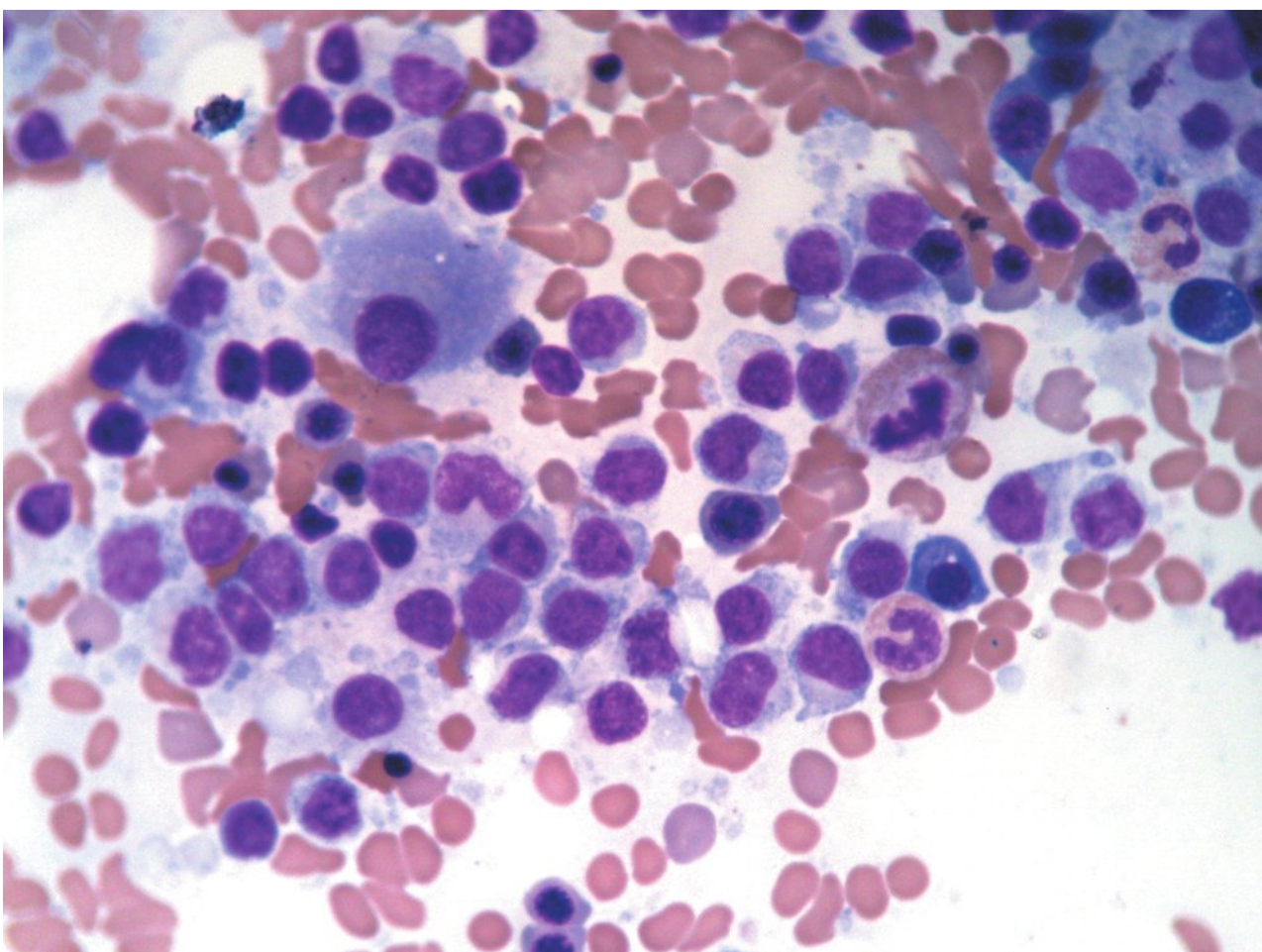


FIGURE 6.25.12 Bone marrow aspirate shows a cluster of leukemic hairy cells intermingled with a few nucleated red cells. Note lymphoid chromatin pattern of the nuclei and the fray cytoplasmic border. Wright–Giemsa, 60 $\times$ magnification.

Another entity that is to be distinguished from HCL and HCLv is splenic diffuse red pulp small B-cell lymphoma (SRPL) (39,51,63). Patients with SRPL had peripheral lymphocytosis with no monocytopenia. This lymphoma involves mainly the red pulp of the spleen with intrasinusoidal infiltration. The tumor cells are small to medium in size with small round nucleus, but, unlike HCLv, small nucleoli are only occasionally seen. Similar to HCL and HCLv, the tumor cells show cytoplasmic projections that are long and large with broad base (39,51,63). This neoplasm is refractory to conventional HCL therapy, but responds well to splenectomy (39,51,63).

HCL, HCLv, and SRPL may represent overlapping entities, but HCLv and SRPL are not considered to be biologically related to HCL and are classified under an umbrella term “splenic B-cell lymphoma, unclassifiable” in the 2008 WHO classification (39).

The treatment of HCL has progressed rapidly in recent years. In a 1994 study, the 5-year survival rate was 34.4% for untreated patients; 58.8% for patients receiving chemotherapy, steroids, or other drugs; 64.1% for splenectomized patients; and 88.9% for interferon- α -treated patients (64). Recently, the drugs of choice are the purine analogues. Both cladribine and pentostatin induce durable complete remissions in the overwhelming majority of patients (2,65,66).

REFERENCES

1. Foucar K, Catovsky D. Hairy cell leukemia. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:138–141.
2. Foucar K, Falini B, Catovsky D, et al. Hairy cell leukemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:188–190.
3. Chang KL, Stroup R, Weiss LM. Hairy cell leukemia. Current status. *Am J Clin Pathol*. 1992;97:719–738.
4. Melo JV, Hedge V, Parreira A, et al. Splenic B-cell lymphoma with circulating villous lymphocytes. Differential diagnosis of B-cell leukemias with large spleens. *J Clin Pathol*. 1987;40:643–651.
5. Melo JV, Robinson DSF, Catovsky D. The differential diagnosis between chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders. Morphological and immunological studies. In: Polliack A, Catovsky D, eds. *Chronic Lymphocytic Leukemia*. Chur, Switzerland: Harwood Academic Publishers; 1988;2:585–602.
6. Katayama I. Bone marrow in hairy cell leukemia. *Hematol Oncol Clin North Am*. 1988;2:585–602.
7. Bitter MA. Hairy cell leukemia. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1531–1555.
8. Cawley JC, Hawkins SF. The biology of hairy-cell leukaemia. *Curr Opin Hematol*. 2010;17:341–349.
9. Cessna MH, Hartung L, Tripp S, et al. Hairy cell leukemia variant: fact or fiction. *Am J Clin Pathol*. 2005;123:132–138.
10. Namba K, Soban EJ, Bowling MC, et al. Splenic pseudosinususes and hepatic angiomatous lesions. Distinctive features of hairy cell leukemia. *Am J Clin Pathol*. 1977;67:415–426.

11. Burks JS, Rappaport H. The diagnosis and differential diagnosis of hairy cell leukemia in bone marrow and spleen. *Semin Oncol*. 1984;11:334–346.
12. Roquest ML, Zafrani E, Farcet JP, et al. Histopathological lesions of the liver in hairy cell leukemia. A report of 14 cases. *Hepatology*. 1985;5:496–500.
13. Sun T, Grupka N, Klein C. Transformation of hairy cell leukemia to high-grade lymphoma: a case report and review of the literature. *Hum Pathol*. 2004;35:1423–1426.
14. Sun T, Susin M. *Differential Diagnosis of Lymphoid Disorders*. New York: Igaku-Shoin; 1996:120–131.
15. Lazzaro B, Munger R, Flick J, et al. Visualization of the ribosome-lamella complex in plastic-embedded biopsy specimens as an aid to diagnosis of hairy cell leukemia. *Arch Pathol Lab Med*. 1991;115:1259–1262.
16. Hounie H, Chittal SM, Saati TA, et al. Hairy cell leukemia. Diagnosis of bone marrow involvement in paraffin embedded biopsy sections with monoclonal antibody DBA-44. *Am J Clin Pathol*. 1992;98:26–33.
17. Forconi F, Sahota SS, Raspadori D, et al. Tumor cells of hairy cell leukemia express multiple clonally related immunoglobulin isotypes via RNA splicing. *Blood*. 2001;98:1174–1181.
18. Forconi F, Sahota SS, Raspadori D, et al. Hairy cell leukemia: at the crossroad of somatic mutation and isotype switch. *Blood*. 2004;104:3312–3317.
19. Miller M, Fishleder AJ, Tubbs RR. The expression of CD22 (Leu 14) and CD11c (Leu M5) in chronic lymphoproliferative disorders using two-color flow cytometric analysis. *Am J Clin Pathol*. 1991;96:100–108.
20. Schwarting R, Stein H, Wang CY. The monoclonal antibodies aS-HCL1 (aLeu-14) and aS-HCL3 (aLeu-M5) allow the diagnosis of hairy cell leukemia. *Blood*. 1985;65:974–983.
21. Vardiman JW, Gilewski TA, Ratain MJ, et al. Evaluation of Leu M5 (CD11c) in hairy cell leukemia by the alkaline phosphatase anti-alkaline phosphatase technique. *Am J Clin Pathol*. 1988;90:250–256.
22. Hanson CA, Gribbin TE, Schnitzer B, et al. CD11c (Leu M5) expression characterizes a B-cell chronic lymphoproliferative disorder with features of both chronic lymphocytic leukemia and hairy cell leukemia. *Blood*. 1990;76:2360–2367.
23. Kristensen JS, Ellegaard J, Hokland P. A two-color flow cytometry assay for detection of hairy cells using monoclonal antibodies. *Blood*. 1987;70:1063–1068.
24. Korsmeyer SJ, Greene WC, Cossman J, et al. Rearrangement and expression of immunoglobulin genes and expression of Tac antigen in hairy cell leukemia. *Proc Natl Acad Sci U S A*. 1983;80:4522–4526.
25. Waldmann TA, Greene WC, Sarin PS, et al. Functional and phenotypic comparison of human T-cell leukemia/lymphoma virus positive adult T-cell leukemia with human T-cell leukemia/lymphoma virus negative Sézary leukemia, and their distinction using anti-Tac. *J Clin Invest*. 1984;73:1711–1718.
26. Hsu SM, Yang K, Jaffe ES. Phenotype expression of Hodgkin's and Reed–Sternberg cells in Hodgkin's disease. *Am J Pathol*. 1985;118:209–217.
27. Garcia CR, Weiss LM, Warnke RA. Small noncleaved cell lymphoma. An immunophenotype study of 18 cases with comparison to diffuse large cell lymphoma. *Hum Pathol*. 1986;17:454–461.
28. Matutes E, Morilla R, Owusu-Ankomah K, et al. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood*. 1994;83:1558–1562.
29. Richard JM, Mick R, Lata JM, et al. Serum soluble interleukin-2 receptor is associated with clinical and pathologic disease status in hairy cell leukemia. *Blood*. 1990;76:1941–1945.
30. Huh YO, Puh WC, Kantarjian HM, et al. Detection of subgroups of chronic B-cell leukemias by FMC-7 monoclonal antibody. *Am J Clin Pathol*. 1994;101:283–289.
31. Falini B, Tiacchi E, Liso A, et al. Simple diagnostic assay for hairy cell leukaemia by immunocytochemical detection of annexin A1 (ANXA1). *Lancet*. 2004;363:1869–1870.
32. Del Giudice I, Matutes E, Morilla R, et al. The diagnostic value of CD123 in B-cell disorders with hairy cell or villous lymphocytes. *Haematologica*. 2004;89:303–308.
33. Jobrens K, Stein H, Anagnostopoulos I. T-bet transcription factor detection facilitates the diagnosis of minimal hairy cell leukemia infiltrates in bone marrow trephines. *Am J Surg Pathol*. 2007;31:1181–1185.
34. Pettitt AR, Zusel M, Cawley JC. Hairy cell leukemia. Biology and management. *Br J Haematol*. 1999;106:2–8.
35. Polliack A. Hairy cell leukemia and allied chronic lymphoid leukemias. Current knowledge and new therapeutic options. *Leuk Lymphoma*. 1997;26(suppl 1):41–51.
36. Chen YH, Tallman MS, Goolsby C, et al. Immunophenotypic variations in hairy cell leukemia. *Am J Clin Pathol*. 2006;125:251–259.
37. Dong HY, Weisberger J, Liu Z, et al. Immunophenotypic analysis of CD103+ B-lymphoproliferative disorders. *Am J Clin Pathol* 2009;131:586–595.
38. Yam LT, Janckila AJ, Li CY, et al. Cytochemistry of tartrate-resistant acid phosphatase. Fifteen years' experience. *Leukemia*. 1987;1:285–288.
39. Piris M, Foucar K, Mollejo M, et al. Splenic B-cell lymphoma/leukaemia, unclassifiable. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:191–193.
40. Matutes E, Wortherspoon A, Brito-Babapulle V, et al. The natural history and clinic-pathological features of the variant form of hairy cell leukemia. *Leukemia*. 2001;15:184–186.
41. Saxon A, Stevens RH, Golde DW. T-lymphocyte variant of hairy cell leukemia. *Ann Intern Med*. 1978;88:323–326.
42. Rosenblatt JD, Golde DW, Wachsman W, et al. A second isolate of HTLV-II associated with atypical hairy cell leukemia. *N Engl J Med*. 1986;315:372–377.
43. Katayama I, Hirashima K, Matuyama K, et al. Hairy cell leukemia in Japanese patients. A study with monoclonal antibodies. *Leukemia*. 1987;1:301–305.
44. Rosenblatt JD, Giorgi JV, Golde DW, et al. Integrated human T-cell leukemia virus II genome in CD8+ T-cells from a patient with “atypical” hairy cell leukemia. Evidence of distinct T and B cell lymphoproliferative disorders. *Blood*. 1988;71:363–369.
45. Van De Corput L, Falkenburg JH, Kluin-Nelemans JC. T-cell dysfunction in hairy cell leukemia: an updated review. *Leuk Lymphoma*. 1998;30:31–39.
46. Went PT, Zimpfer A, Pehrs AC, et al. High specificity of combined TRAP and DBA-44 expression for hairy cell leukemia. *Am J Surg Pathol*. 2005;29:474–478.
47. Hoyer JD, Li CY, Yam LT, et al. Immunohistochemical demonstration of acid phosphatase isoenzyme 5 (tartrate-resistant) in paraffin sections of hairy cell leukemia and other hematologic disorders. *Am J Clin Pathol*. 1997;108:308–315.
48. Raghavachar A, Bartram CR, Porzsolt F. Eradication by alpha-interferon of one clone in biclonal hairy cell leukemia. *Lancet*. 1986;2:516.

49. Hockley SL, Giannouli S, Morilla A, et al. Insight into the molecular pathogenesis of hairy cell leukaemia, hairy cell leukaemia variant and splenic marginal zone lymphoma, provided by the analysis of their IGH rearrangements and somatic hypermutation patterns. *Br J Haematol*. 2009;148:666–669.
50. Arons E, Suntum T, Stetler-Stevenson M, et al. VH4-34 hairy cell leukemia, a new variant with poor prognosis despite standard therapy. *Blood*. 2009;114:4687–4695.
51. Traverse-Glehen A, Baseggio L, Callet-Bauchu E, et al. Hairy cell leukaemia-variant and splenic red pulp lymphoma: a single entity? *Br J Haematol*. 2010;150(1):113–116.
52. Forconi F, Sozzi E, Cencini E, et al. Hairy cell leukemia with unmutated IGHV genes define the minor subset refractory to single-agent cladribine and with more aggressive behavior. *Blood*. 2009;114:4696–4702.
53. Vanhenterrijk V, De Wolf-Peeters C, Wlodarska I. Comparative expressed sequence hybridization studies of hairy cell leukemia show uniform expression profile and imprint of spleen signature. *Blood*. 2004;104:250–255.
54. Basso K, Lisa A, Tiacci E, et al. Gene expression profiling of hairy cell leukemia reveals a phenotype related to memory B cells with altered expression of chemokine and adhesion receptors. *J Exp Med*. 2004;199:59–68.
55. Sadamori N, Han T, Block AW, et al. Cytogenetic studies of stimulated lymphocytes in hairy cell leukemia. *Cancer Genet Cytogenet*. 1985;17:69–74.
56. Brito-Babapulle V, Pittman S, Melo JV, et al. The 14q+ marker in hairy cell leukemia. A cytogenetic study of fifteen cases. *Leuk Res*. 1986;10:131–138.
57. Hoffman MA. Clinical presentations and complications of hairy cell leukemia. *Hematol Oncol Clin N Am*. 2006;20:1065–1073.
58. Sainati L, Matutes E, Mulligan S, et al. A variant form of hairy cell leukemia resistant to α -interferon. Clinical and phenotypic characteristics of 17 patients. *Blood*. 1990;76:157–162.
59. Sun T, Susin M, Shevde N, et al. Hybrid form of hairy cell leukemia. *Hematol Oncol*. 1990;8:283–294.
60. Wormsley SB, Baird SM, Gadol N, et al. Characteristics of CD11c+ CD5+ chronic B-cell leukemias and the identification of novel peripheral B-cell subsets with chronic lymphoid leukemia immunophenotypes. *Blood*. 1990;76:123–130.
61. Caligaris-Cappio F, Pizzolo G, Chilosi M, et al. Phorbol ester induces abnormal chronic lymphocytic leukemia cells to express features of hairy cell leukemia. *Blood*. 1985;66:1035–1042.
62. Ziegler-Heitbrock HWL, Munker R, Dorken BM, et al. Induction of features characteristic of hairy cell leukemia in chronic lymphocytic leukemia and prolymphocytic leukemia cells. *Cancer Res*. 1986;46:2172–2178.
63. Traverse-Glehen A, Baseggio L, Callet-Bauchu E, et al. Splenic red pulp lymphoma with numerous basophilic villous lymphocytes: a distinct clinicopathologic and molecular entity? *Blood*. 2008;111:2253–2260.
64. Frassoldti A, Lamparelli T, Federico M, et al. Hairy cell leukemia. A clinical review based on 725 cases of the Italian Cooperative Group (ICGHCL). Italian Cooperative Group for Hairy Cell Leukemia. *Leuk Lymphoma*. 1994;13:307–316.
65. Tallman MS, Peterson LC, Hakimian D, et al. Treatment of hairy-cell leukemia. Current views. *Semin Hematol*. 1999;36:155–163.
66. Cannon T, Mobarek D, Wegge J, et al. Hairy cell leukemia: Current concepts. *Cancer Invest*. 2008;26:860–865.

CASE 26

Splenic B-Cell Marginal Zone Lymphoma

CASE HISTORY

A 46-year-old woman presented with weakness, fever, and left flank tenderness. She had a long history of Coombs-positive hemolytic anemia, requiring frequent transfusions. Physical examination showed marked splenomegaly but no hepatomegaly or lymphadenopathy. Laboratory data revealed a hemoglobin level of 7.1 g/dL, hematocrit 21%, platelets 200,000/mL, and reticulocyte count 19.6%. The leukocyte count was 5,800/mL with 64% lymphocytes. A direct Coombs test was positive. Serum electrophoresis showed no monoclonal gammopathy. The patient was initially treated with steroids and subsequently underwent a splenectomy. Her hemoglobin and hematocrit promptly returned to normal. She became asymptomatic and returned to work fulltime 1 year after splenectomy.

FLOW CYTOMETRIC FINDINGS

Peripheral blood: IgG 3%, IgA 2%, IgM 64%, k 3%, l 66%, CD19 65%, CD20 70%, HLA-DR 64%, CD3 28%, CD5 29%, CD7 28%, CD11c 32%, CD14 17% (Fig. 6.26.1).

Bone marrow: IgG 2%, IgA 1%, IgM 56%, k 2%, l 58%, CD19 67%, CD20 63%, HLA-DR 64%, CD3 32%, CD4 18%, CD5 28%, CD7 30%, CD8 13%, CD11c 25%, CD25 0%.

Spleen: IgG 4%, IgA 2%, IgM 52%, k 9%, l 45%, CD3 36%, CD5 34%, CD7 36%, CD11c 20%, CD25 2%.

DISCUSSION

Splenic marginal zone lymphoma (SMZL) has been reported under various names, such as splenomegalic immunocytoma (1,2), malignant lymphoma–simulating

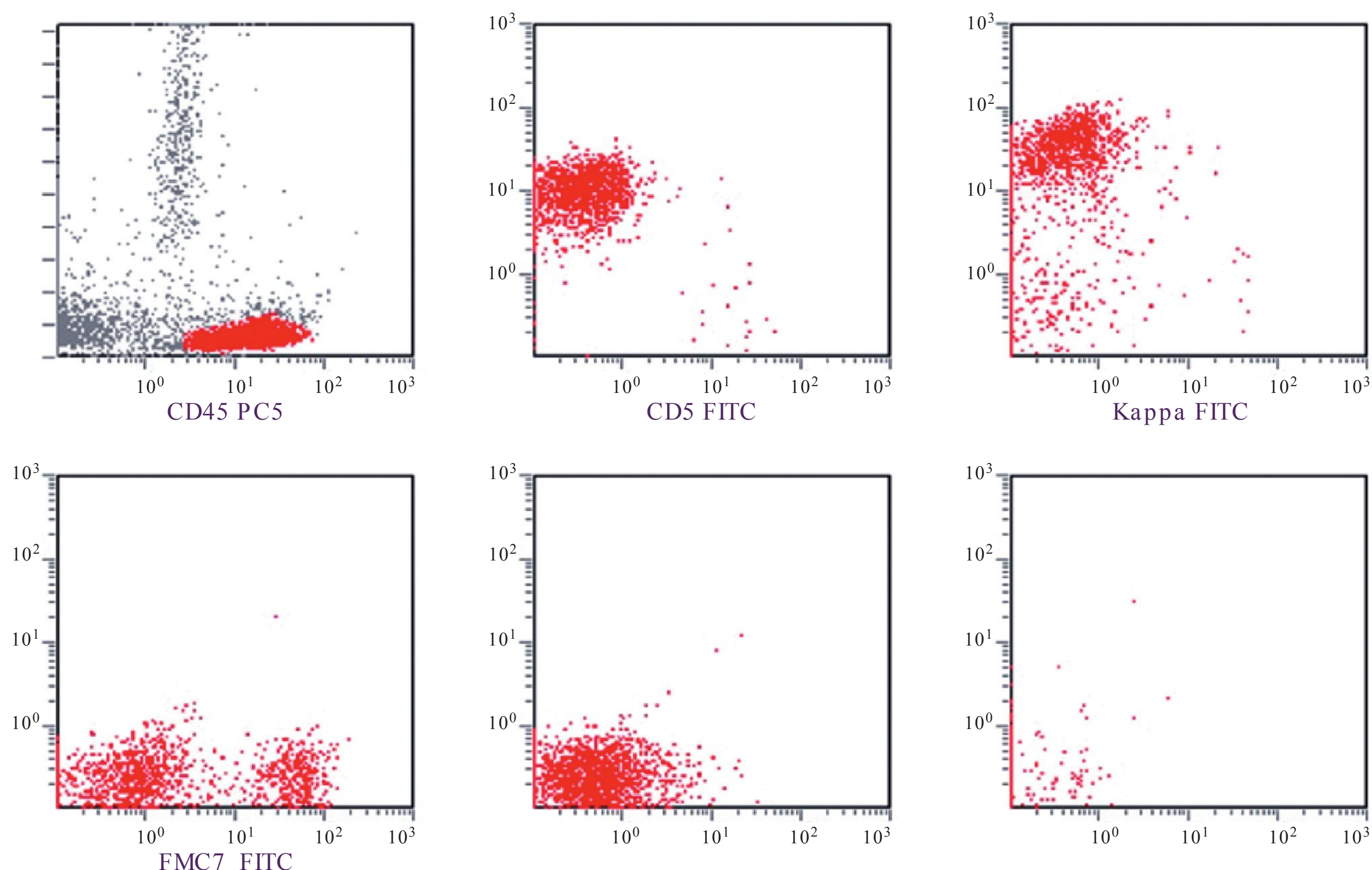


FIGURE 6.26.1 Flow cytometry histograms show positive CD19 and FMC-7 markers in a monoclonal B-cell population. CD5, CD23, and CD10 are negative, excluding mantle cell lymphoma, small lymphocytic lymphoma, and follicular lymphoma. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

leukemic reticuloendotheliosis (3), lymphocytic lymphoma–simulating hairy cell leukemia (HCL) (4), and chronic lymphoproliferative disorder resembling HCL (5). Melo et al. (6,7) first applied the term splenic lymphoma with villous lymphocytes (SLVL) in 1987. The terminology was adopted by the French-American-British (FAB) cooperative group in 1989 (8). Schmid et al. (9) first used the term SMZL, and the same group confirmed that SLVL and SMZL were similar histologically and immunophenotypically (10). Subsequently, SLVL was classified as SMZL as a provisional entity in the revised European–American classification of lymphoid neoplasms (11). This term has been adopted by the World Health Organization (WHO) classification of lymphoid neoplasms (12). In the 2008 WHO classification, SMZL is designated splenic B-cell marginal zone lymphoma (13).

Morphology

SMZL is defined as a small B-cell lymphoma involving the spleen with a characteristic feature in the white pulp, variably described as bizonal or biphasic phenomenon or margination (Fig. 6.26.2; Table 6.26.1). The germinal center of the white pulp is surrounded and finally replaced by small round lymphocytes (10,12,14). The small lymphocytes are, in turn, surrounded by medium-sized lymphocytes with more dispersed chromatin and abundant pale cytoplasm

resembling the marginal zone cells. Scattered transformed large lymphoid cells are also seen in the outer zone. The mantle zone is effaced. Dunn-Walters et al. (15) believed that both small and large cells are of the same neoplastic clone, which is in contrast to the original thinking that the central small cells are the preserved mantle (9). When the germinal center is preserved, follicular colonization, manifested as neoplastic infiltration of the germinal center, can be observed (16). A monophasic pattern composed of pure marginal zone–like cells has been recognized in recent years (13,17). However, patients with biphasic or monophasic pattern show similar clinicopathologic features and survival (17).

In the red pulp, besides sinusoidal infiltration, small nodules are sometimes present with the composition of tumor cells similar to that in the periphery of the white pulp follicles (10). Lymphoplasmacytic differentiation may be present, but the frequency is variable in different reports (6,16,18).

There are also many splenic small B-cell lymphomas that mimic SMZL and are now classified as splenic B-cell lymphoma/leukemia, unclassifiable in the 2008 WHO classification (19). This new category includes two provisional entities: splenic diffuse red pulp small B-cell lymphoma and HCL variant. The distinction between these two entities and SMZL as well as HCL is discussed under Case 25 Hairy Cell Leukemia.

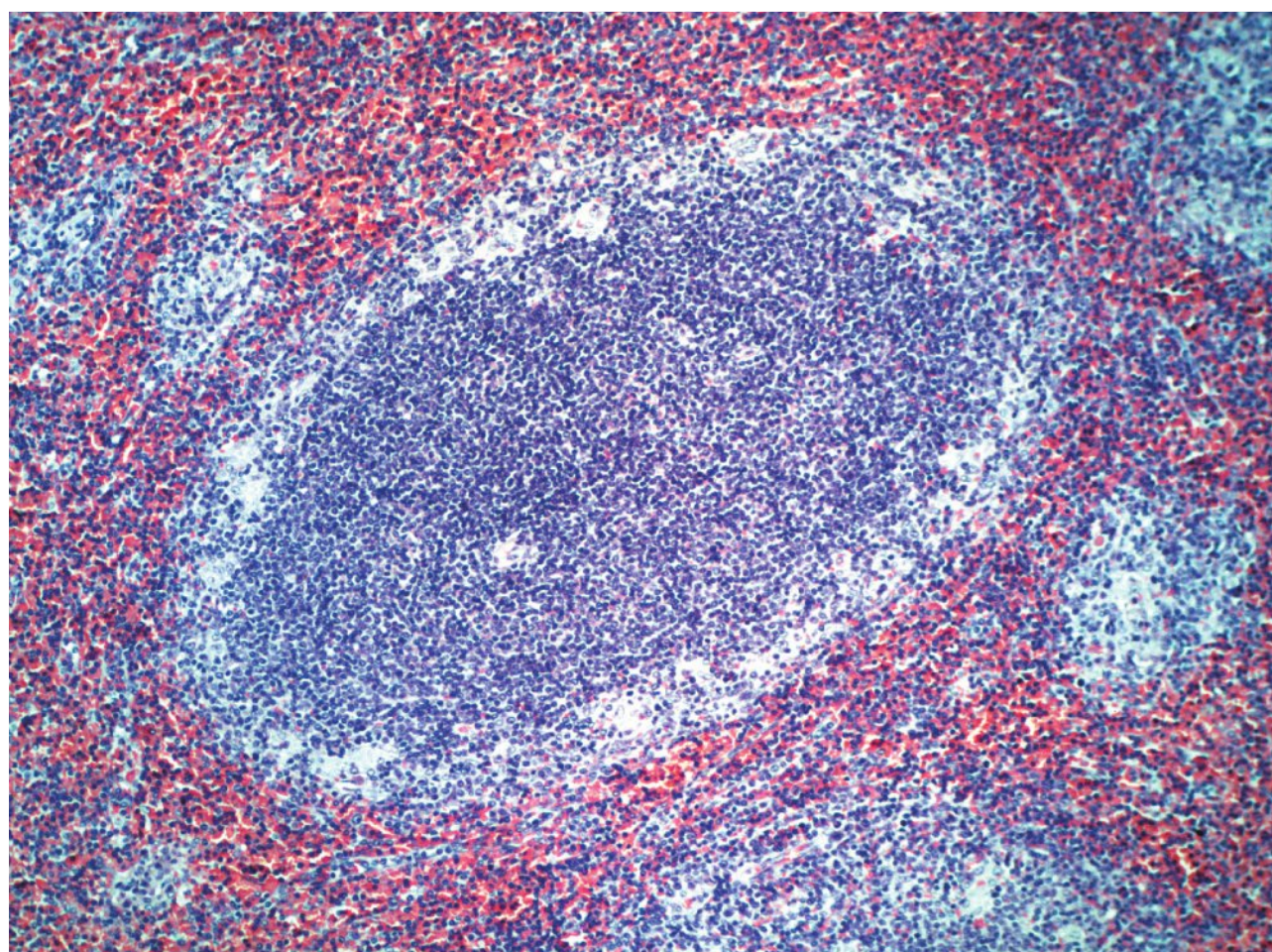


FIGURE 6.26.2 Splenectomy specimen reveals the bizonal phenomenon in the white pulp with the small cells in the center, replacing the germinal center and the marginal zone cells with pale cytoplasm in the periphery. Several cellular nodules composed of both cell types are seen in the red pulp around the follicle. Hematoxylin and eosin, 10× magnification.

In SMZL, peripheral lymph node is seldom involved, but the involvement of the splenic hilar lymph node is more frequent (10,12). In the lymph node, the characteristic feature is a micronodular pattern, without the presence of marginal differentiation (Fig. 6.26.3) (12,20). However, the small lymphoid tumor cells and the marginal zone cells are still present in the lymph node and are intermixed. The sinuses are generally dilated. In the earlier stage, pathology may be confined to the cortical and paracortical areas, but diffuse effacement can be seen in later stages (2,3,18,21,22).

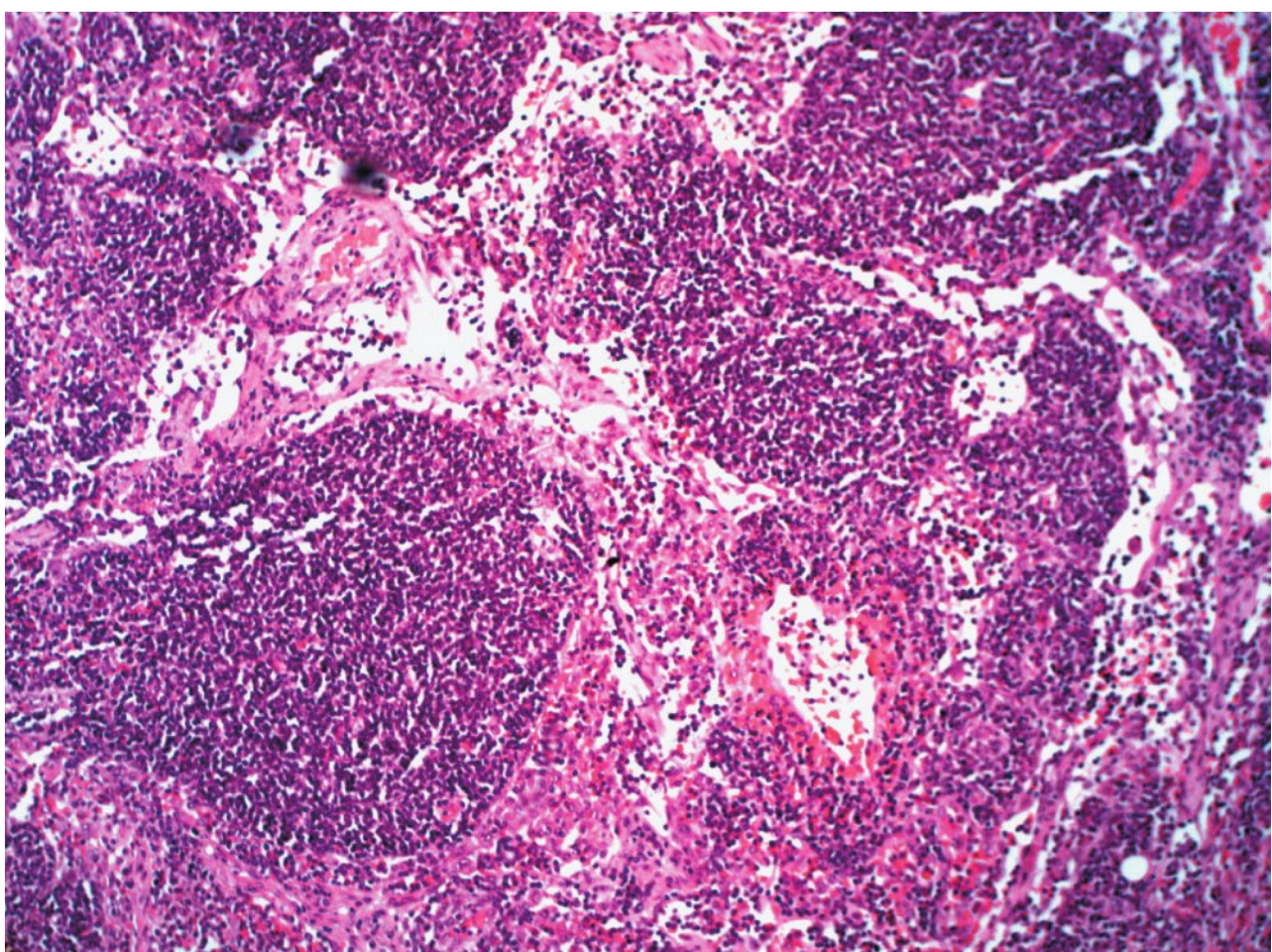


FIGURE 6.26.3 Splenic hilar lymph node biopsy shows a micronodular pattern with dilated sinuses. Hematoxylin and eosin, 10× magnification.

The bone marrow is frequently involved in SMZL, accounting for 90% to 96% in one study (Boveri). The infiltration can be nodular, interstitial, diffuse, or paratrabecular (Fig. 6.26.4) (2,3,6,18,21,23). However, the most characteristic pattern is intrasinusoidal infiltration, and the demonstration of this pattern may be facilitated by immunohistochemical stains with CD20, CD22, or DBA-44 (13,24). In an appropriate clinical setting, this pattern is considered by some to be diagnostic for SMZL and makes splenectomy unnecessary for diagnostic purposes (25–27). Hepatosplenic T-cell lymphoma may also show sinusoidal infiltration in the bone marrow, but the immunophenotype is entirely different. The nodular pattern is most common (88%), but the intrasinusoidal pattern coexistent with other patterns is seen in 70% cases in one study series (28). Similar results were observed in another study (29).

TABLE 6.26.1	
Characteristic Morphologic Features of SMZL	
Histologic pattern	Mainly splenic white pulp involvement with bizonal phenomenon; tumor nodules present in the red pulp with sinusoidal infiltration; sinusoidal infiltration in the bone marrow; micronodular pattern in the lymph node
Cytology	Small, darkly stained lymphoid cells in the center and pale stained large cells in the periphery of splenic follicles; villous lymphocytes in the peripheral blood
Specific features	Nodular pattern and bizonal phenomenon in the spleen; sinusoidal infiltration in the bone marrow

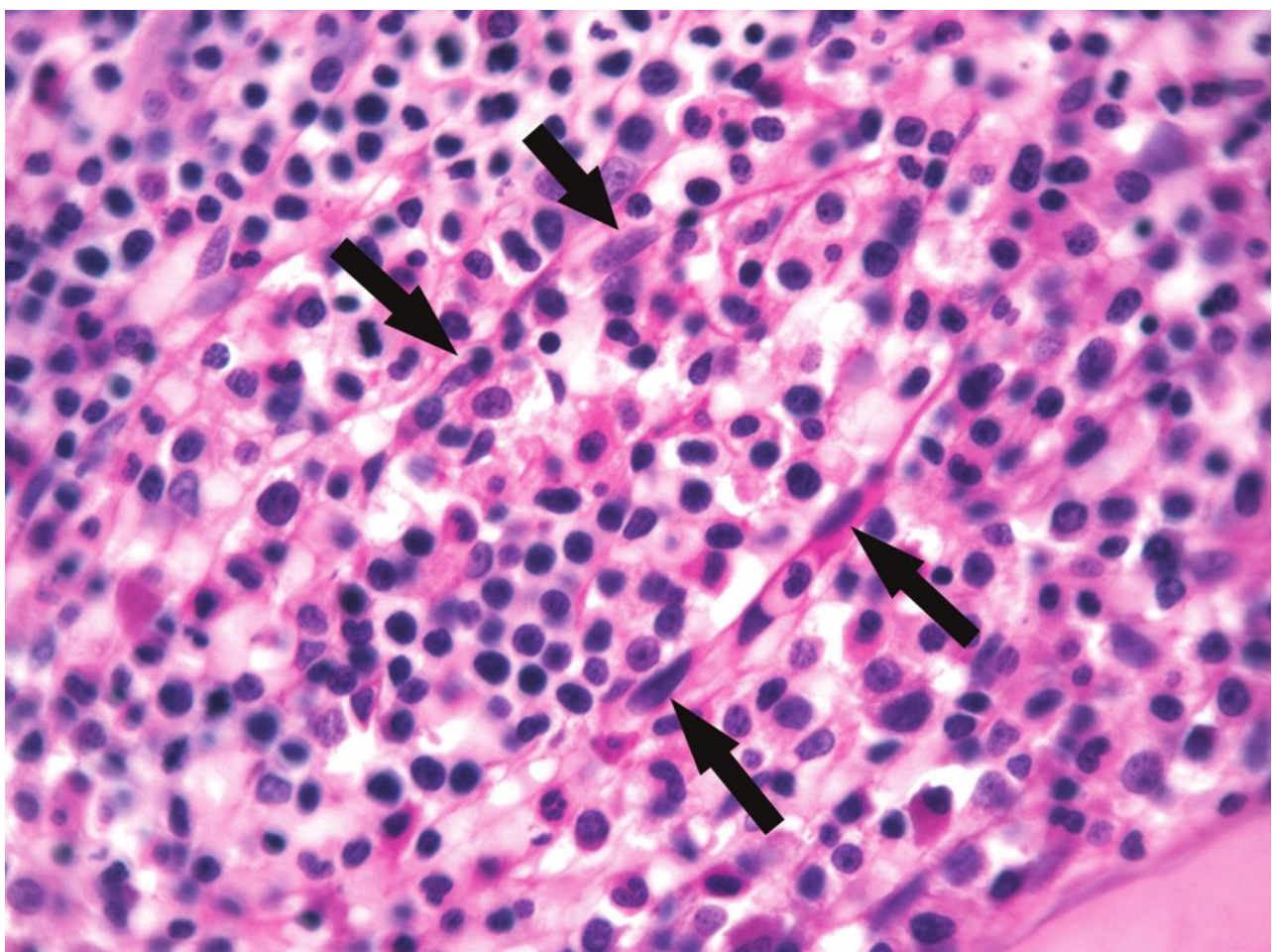
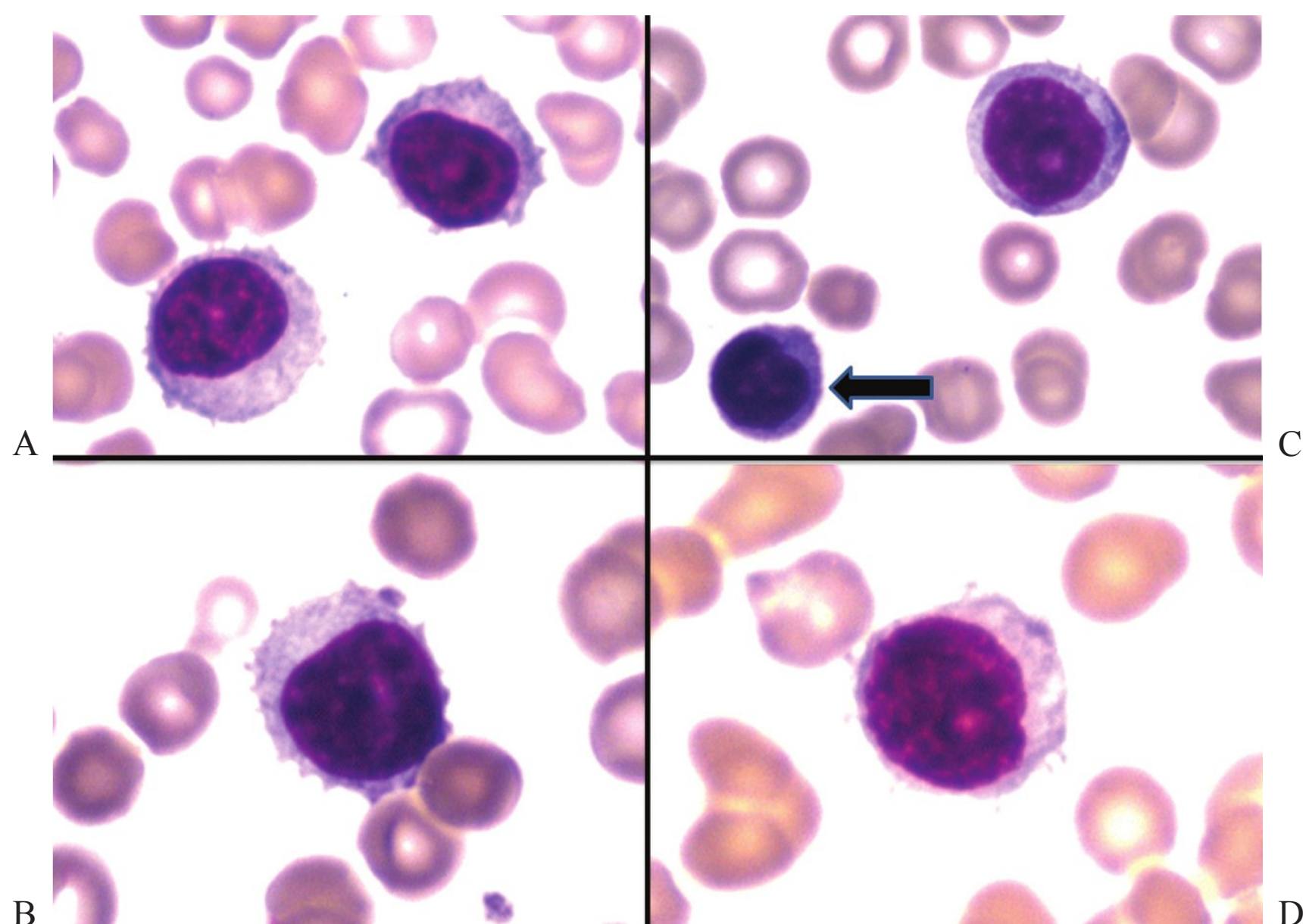


FIGURE 6.26.4 Bone marrow biopsy shows lymphoid infiltration replacing the normal hematopoietic cells. Note the endothelial lining of the marrow sinuses (black arrows), which highlights the intrasinusoidal infiltration. Hematoxylin and eosin, 60× magnification.

FIGURE 6.26.5 Villous lymphocytes of various shapes demonstrated in peripheral blood smear as compared with a normal lymphocyte (arrow). Wright–Giemsa, 100× magnification.



In the peripheral blood, villous lymphocytes may or may not be present in SMZL. However, in SLVL, villous lymphocytes constitute 38% to 99% of all lymphocytes present in the peripheral blood (30). Some authors advocate the use of 20% villous lymphocytes as the cutoff point between SLVL and SMZL (31). Nevertheless, as these two diseases show no significant difference in their clinical features, most studies consider them to be the same entity.

A typical villous lymphocyte is larger than the small lymphocytes found in chronic lymphocytic leukemia (CLL) and generally smaller than prolymphocytes (6,7,30). It has a round or ovoid nucleus with clumped chromatin; in one half of cases, it possesses a small but distinct nucleolus. The cytoplasm is usually moderate and basophilic, but it may be scanty in some cases. However, the striking feature of this cell is the presence of thin and short cytoplasmic villi with polar distribution (Fig. 6.26.5). In contrast, hairy cells usually have numerous slender cytoplasmic villi (hairy projections) and a uniformly dispersed chromatin pattern. The large size and lower nucleocytoplasmic ratio of hairy cells also help to distinguish them from the villous lymphocytes of SLVL. The small-cell variant of SLVL may show scanty cytoplasm and no nucleoli (30); in those cases, CLL should be excluded by marker studies.

Electron microscopy may show a polar distribution of a small number of short and thin cytoplasmic villi in cells of SLVL (Fig. 6.26.6), whereas cells from HCL may show ribosome–lamellar complex and evenly distributed long and slender cytoplasmic projections (Fig. 6.26.7).

In the liver, the portal tracts are mainly involved with nodular infiltration, but sinusoidal infiltration is also present (2,3,19,21,26). Other organs, such as skin, lung, and meninges, have also been reported in a few cases (27). Transformation to large-cell lymphoma has been reported in 5% of SLVL cases (14) but in 10% to 13% of SMZL cases (25,31).

The major differential diagnoses of lymphomas involving the spleen include HCL, CLL, prolymphocytic leukemia, and

mantle cell lymphoma. HCL involves mainly the red pulp with characteristic pseudosinus formation. Prolymphocytic leukemia shows the bizonal phenomenon similar to SMZL, but the cytology and immunophenotype should help distinguish these two entities. Mantle cell lymphoma is more difficult to distinguish from SMZL, but it involves mainly the white pulp, the tumor cells are monotonous, and plasma cell differentiation

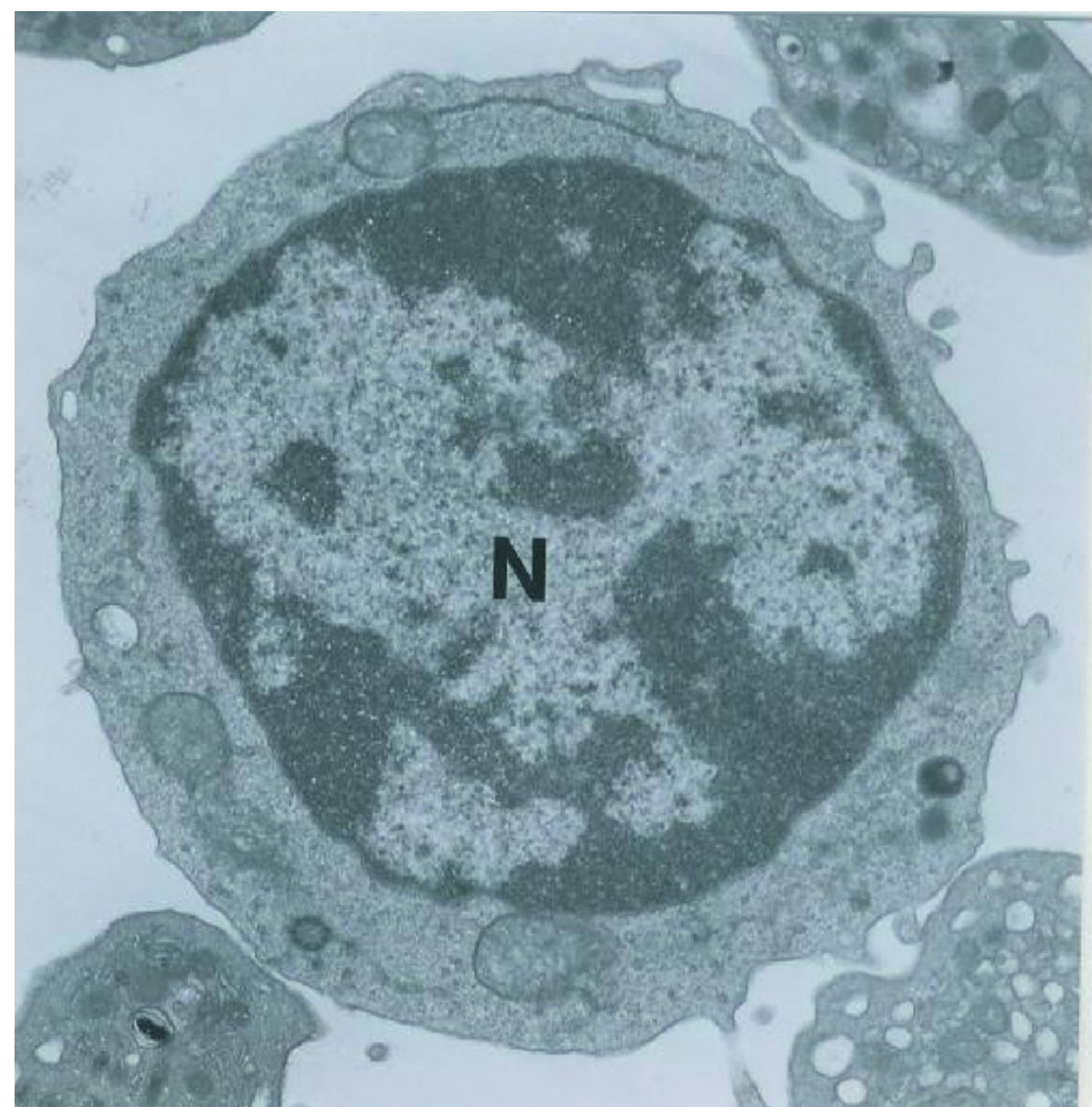


FIGURE 6.26.6 Electron micrograph shows a villous lymphocyte with polar distribution of cytoplasmic projection and no ribosome–lamellar complex. N, nucleus. 13,000× magnification. (Courtesy of Dr. Saul Teichberg, North Shore University Hospital, New York.)

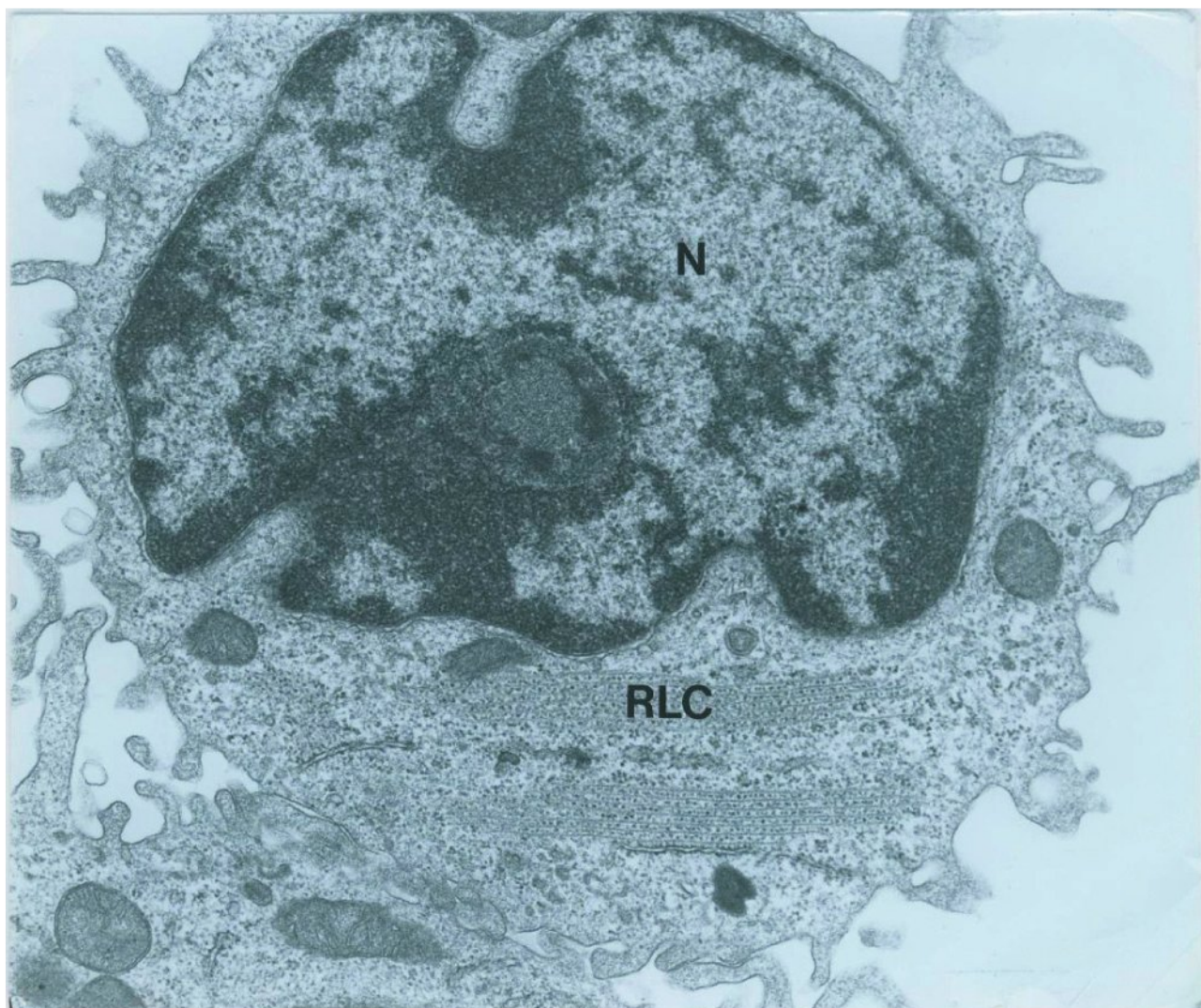


FIGURE 6.26.7 Electron micrograph shows a hairy cell with many fairly uniformly distributed long and slender cytoplasmic projections and two ribosome–lamellar complexes (RLCs). N, nucleus. 25,000× magnification. (Courtesy of Dr. Saul Teichberg, North Shore University Hospital, New York.)

is absent (16). CLL involves both the red pulp and white pulp, and the immunophenotype differs from that of SMZL.

Immunophenotype

Many markers have been studied in cases of SMZL and SLVL. The tumor cells are positive for surface Igs, CD19, CD20, CD22, CD24, FMC-7, HLA-DR, and CD45 (Table 6.26.2) (10,14,16,18,23,31–33). Immunohistochemical stains may demonstrate DBA-44, CD79a, CD79b, bcl-2, paired box-containing family of genes/B-cell specific activator protein (PAX/BSAP) and CD45RA/MT2 (10,14,24,26,34,35). Several immunohistochemical negative markers (CD10, CD23, CD43, bcl-6, and cyclin D1) are helpful in differential diagnosis (26).

In a study of 157 marginal zone lymphomas, DBA-44 was demonstrated in 38% of SMZL cases, but only in 4% each of nodal and extranodal marginal zone lymphoma cases (29). DBA-44 stain is predominantly seen in the

diffuse infiltration pattern in the red pulp (29). One study emphasized that cases with a monophasic histologic pattern were frequently IgD negative and CD43 positive (17).

HCL is characterized by its expression of CD11c, CD25, FMC-7, CD103, HC2, and tartrate-resistant acid phosphatase, and these markers used to be considered helpful to distinguish HCL, SMZL, and SLVL. However, current studies have found that one or more of these markers can be positive in some cases of SMZL and/or SLVL, but that only CD103, CD123, Annexin A1, and HC2 are specific enough to make a definitive diagnosis of HCL (10,13,33). However, in SMZL, not all HCL markers are present in the same case (27).

SMZL and SLVL are usually negative for CD5 and CD23, which can help to distinguish small lymphocytic lymphoma and CLL (CD5+, CD23+) and mantle cell lymphoma (CD5+, CD23–) (14,16,24,32,34). Mantle cell lymphoma is also positive for cyclin D1 and CD43, but both markers are negative in SMZL (26). However, CD5 and CD23 can be positive in 20% to 30% of SMZL/SLVL cases (14). CD5-positive cases differ from CD5-negative cases in higher lymphocyte count, more frequent diffuse bone marrow infiltration, and more frequent mutated Ig heavy-chain variable (IGHV) gene status (36). It is controversial whether these two groups have different cytogenetic makeup (37).

Similar to SMZL, prolymphocytic leukemia is positive for FMC-7 and B-cell markers, but negative for CD23. CD5 is positive in one third of prolymphocytic leukemia cases (7); these cases are most likely transformed from CLL.

The immunohistochemical stains for CD10 and CDw75 (LN1) are usually negative in SMZL and SLVL cases, which distinguish them from follicular lymphoma (10,16,32,37). Although bcl-2 protein can be present in SMZL/SLVL, and follicular lymphoma, BCL-2 gene rearrangement is not detected in the former (10,32,35,38). The negative reaction to bcl-6 in immunohistochemical stain in SMZL and SLVL cases also helps to distinguish them from follicular lymphoma (26).

The other negative markers in SMZL and SLVL include CD2, CD3, CD14, CD35, CD43, CD45RO, and CD68 (10,32). CD38 can be present in 30% to 38% of SLVL and SMZL cases (10,33). The expression of CD38 usually predicts an unfavorable prognosis (39).

The salient features for laboratory diagnosis of SMZL are summarized in Table 6.26.3.

TABLE 6.26.2												
Marker Differences between SMZL and Other B-cell Lymphomas												
	CD5	CD10	CD11c	CD19	CD20	CD22	CD23	CD25	FMC-7	bcl-1	bcl-2	bcl-6
SMZL	–	–	±	+	+	+	–	±	+	–	+	–
HCL	–	–	+	+	+	+	–	+	+	+	–	–
SLL	+	–	–	+	+	–	+	–	–	–	–	–
PLL	±	–	–	+	+	±	–	–	+	–	–	–
MCL	+	–	–	+	+	+	–	–	+	+	+	–
FL	–	+	–	+	+	+	–	–	–	–	+	+

FL, follicular lymphoma; HCL, hairy cell leukemia; MCL, mantle cell lymphoma; PLL, prolymphocytic leukemia; SMZL, splenic marginal zone lymphoma; SLL, small lymphocytic lymphoma.

TABLE 6.26.3

Salient Features for Laboratory Diagnosis of SMZL

1. Mild-to-moderate peripheral lymphocytosis is present with or without villous lymphocytes.
2. The villi on tumor cells are thin and short with polar distribution.
3. Positive B-cell antigens: IgD, CD19, CD20, CD22, CD24, CD79a, CD79b, bcl-2, FMC-7, HLA-DR
4. Specific markers: CD11c, CD25, and tartrate-resistant acid phosphatase are positive in some cases.
5. Negative CD103, CD123, Annexin A1, and HC2 in SMZL are helpful to distinguish from HCL.
6. Peripheral plasmacytosis and monoclonal gammopathy may be present.

HCL, hairy cell leukemia; SMZL, splenic marginal zone lymphoma.

Comparison between Flow Cytometry and Immunohistochemistry

A diagnostic immunophenotype may not be demonstrated by either flow cytometry or immunohistochemistry. However, some cases of SMZL lymphoma may be positive for CD11c and CD25, so that may help distinguish other lymphomas besides HCL. Immunohistochemistry has the advantage of correlating morphology and immunophenotype; therefore, it is usually more useful in differential diagnosis. Since lymphoid hyperplasia in the spleen also shows CD20-positive enlarged follicles, it is the demonstration of positive staining in the periarterial sheath area (normally T-cell zone) that helps the diagnosis of atypical cases (Fig. 6.26.8).

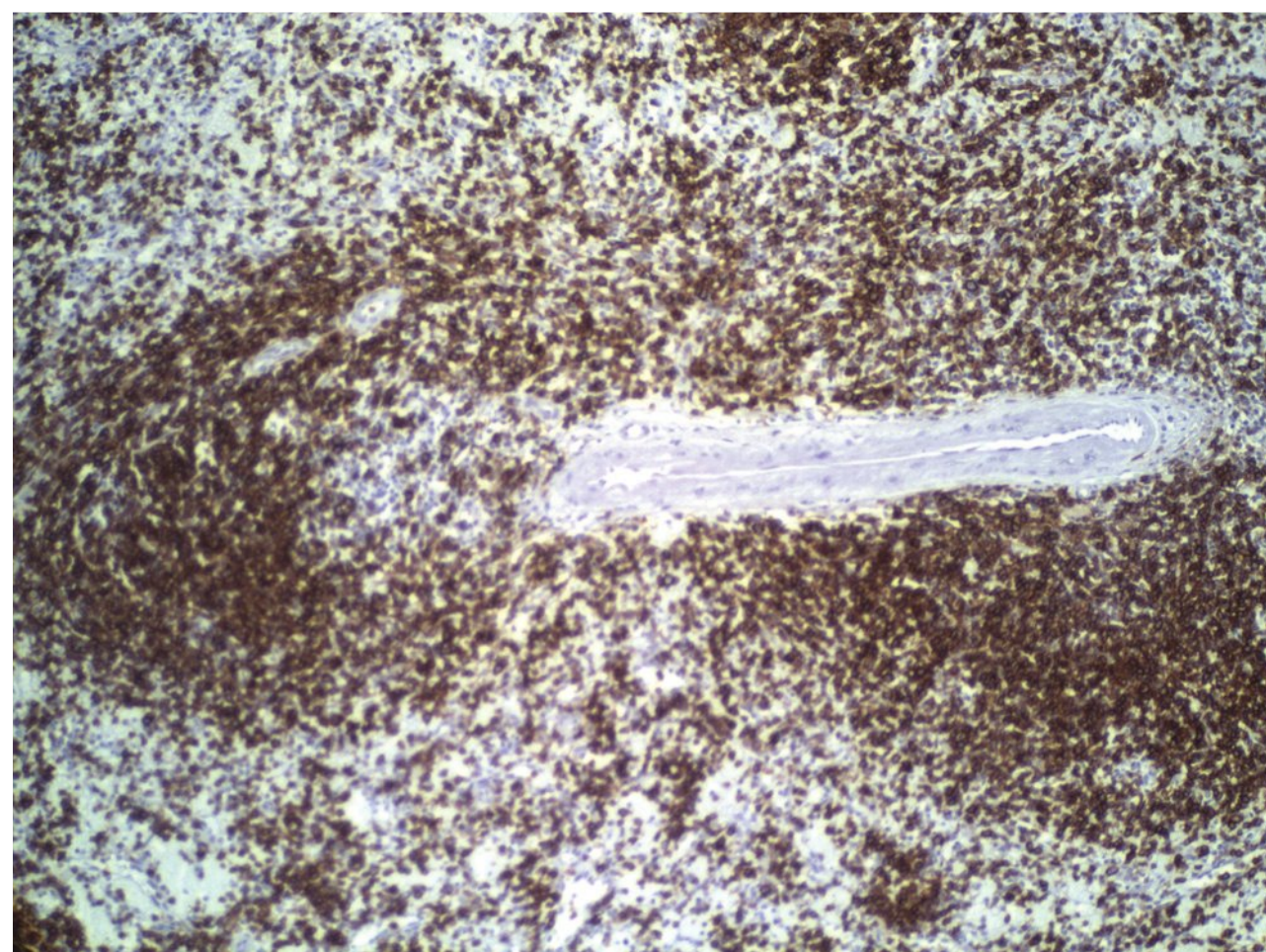


FIGURE 6.26.8 Splenectomy specimen shows CD20 staining of lymphoid cells in the follicle and periarterial sheath, representing tumor cells spreading. Immunoperoxidase, Hematoxylin and eosin, 10× magnification.

Molecular Genetics

As a B-cell lymphoma, Ig gene rearrangement has been demonstrated in SMZL and SLVL cases, but T-cell receptor b-chain gene and bcl-2 are in germlines (32). The demonstration of Ig gene rearrangement by polymerase chain reaction in the spleen may help to distinguish a malignant from a benign lesion (Fig. 6.26.9). Of more interest is the mutation status of the Ig heavy-chain gene. Earlier literature considered that SMZL originated from postgerminal center memory B cells because the tumor cells have mutated IGHV genes (15). However, recent studies have found that SMZL cases may also have unmutated IGHV genes and positive surface IgD that are consistent with a naïve B-cell origin (40). These two cell populations (memory and naïve B cells) are normally present in the splenic marginal zone. Cases with unmutated IGHV genes were reported to have a more aggressive clinical course (41). However, recent studies have found that unmutated IGHV status does not have an impact in overall survival of the patients (37). In addition, several studies have demonstrated the nonrandom use of IGHV gene segments (IGHV1-2) in SMZL cases (37,40). This finding suggests that at least some cases of SMZL are antigen driven, probably due to infections. Indeed, SMZL cases associated with malarial or hepatitis C virus infections have been reported (42,43).

In a study of 330 SMZL cases, 72% revealed cytogenetic aberrations with 53% showing a complex karyotype (Fig. 6.26.10). The most common cytogenetic abnormality in SMZL involves allelic loss at 7q21-32, or translocation involving this region and the k-chain region on chromosome 2, accounting for 40% to 45% of cases (20,27,31). As a result, the CDK6 gene located on 7q22 may be deregulated and might contribute to the pathogenesis of SMZL. For numeric abnormalities, whole or partial trisomy 3 is the most frequent finding in marginal zone B-cell lymphomas, accounting for 36% in SMZL (14,27,31).

In a study of 330 SMZL cases, the predominant aberrations were gain of chromosomes 3/3q and 12q, deletion of 7q and 6q, and translocation involving 8q/1q/14q (37). A study with comparative genomic hybridization of 34 SMZL patients, in which 12 cases were hepatitis C virus positive, showed that the most frequent copy number alterations involved chromosomes 7 and 17 (44). Except for Xp gain, no differences in common alterations were found between hepatitis C virus-positive and hepatitis C virus-negative cases (44).

There are many cytogenetic abnormalities reported, but the most controversial is t(11;14)(q13;q32), which has been described variably in 15% to 26% of patients with SMZL (20,31,45,46). t(11;14) is mainly seen in mantle cell lymphoma, representing bcl-1/IgH translocation, and bcl-1 encodes cyclin D1 or CCND1 protein. However, in the series of Troussard et al. (45), only 8 of 30 cases expressed CCND1, and 1 of 62 cases had bcl-1/IgH rearrangement. Cyclin D1 was not demonstrated by an immunoperoxidase technique in all 9 and 17 cases of SMZL in two series, respectively (35,47). Therefore, these cases are probably not misdiagnosed mantle cell lymphoma. As the breakpoints at 11q13 differ between SMZL and mantle cell lymphoma, molecular biologic analysis may furnish a correct diagnosis (45).

Gene expression profiling and tissue immunohistochemical microarray provide promising results for stratification



FIGURE 6.26.9 Polymerase chain reaction with capillary electrophoresis technique reveals a monoclonal peak in each of the three frameworks, representing immunoglobulin heavy-chain gene rearrangement. (From Sun T. Atlas of Hematologic Neoplasms, Springer, 2009).

of different patient subgroups in terms of prognosis (39). Shorter survival was associated with CD38 expression, naïve IGHV genes, and the expression of a set of nuclear factor-κB genes (39). In addition, gene profiling displays a largely homogenous signature, implying the existence of a single molecular entity (39).

Clinical Manifestations

SMZL is a low-grade non-Hodgkin lymphoma with a stable or slowly progressive clinical course (26,27,31,48). Most patients are older than 50 years. The presenting symptoms are usually mild weakness, fatigue, and abdominal discomfort caused by the enlarged spleen (6). Mild anemia and thrombocytopenia are common at presentation, usually due to hypersplenism (27).

Lymphocytosis, ranging from 10,000 to 40,000/mL, is a consistent feature of SMZL that is helpful to distinguish it from HCL, which often shows cytopenia in the peripheral blood. In addition to the circulating villous lymphocytes, 3% to 12% of plasma cells or plasmacytoid cells may be present in the peripheral blood in SMZL/SLVL (30). Monoclonal

gammopathy is also frequently demonstrated, varying from 30% to 60% of cases (6,26,27,31). In some small series, monoclonal gammopathy was reported to be rare (18,32). Plasmacytosis and monoclonal gammopathy, if present, are useful but not specific for differential diagnosis.

In about 10% of patients, autoimmune phenomenon may be the presenting feature. Immune hemolytic anemia, immune thrombocytopenia, rheumatoid arthritis, cold agglutinins, anticardiolipin antibodies, lupus anticoagulant, and acquired von Willebrand disease have been reported in SMZL cases (26,27,31).

Asymptomatic patients with mild lymphocytosis but without cytopenia need only to have close follow-up (14,20,26,27,31). This group of patients may survive 10 to 15 years without treatment (14). When splenomegaly causes marked discomfort and/or hypersplenism, splenectomy is the treatment of choice (2,20,26,27,31,48). Most patients may have a long remission period after splenectomy. Only when patients have a progressive clinical course or when the disease has transformed into a large-cell lymphoma should chemotherapy be considered. A French study

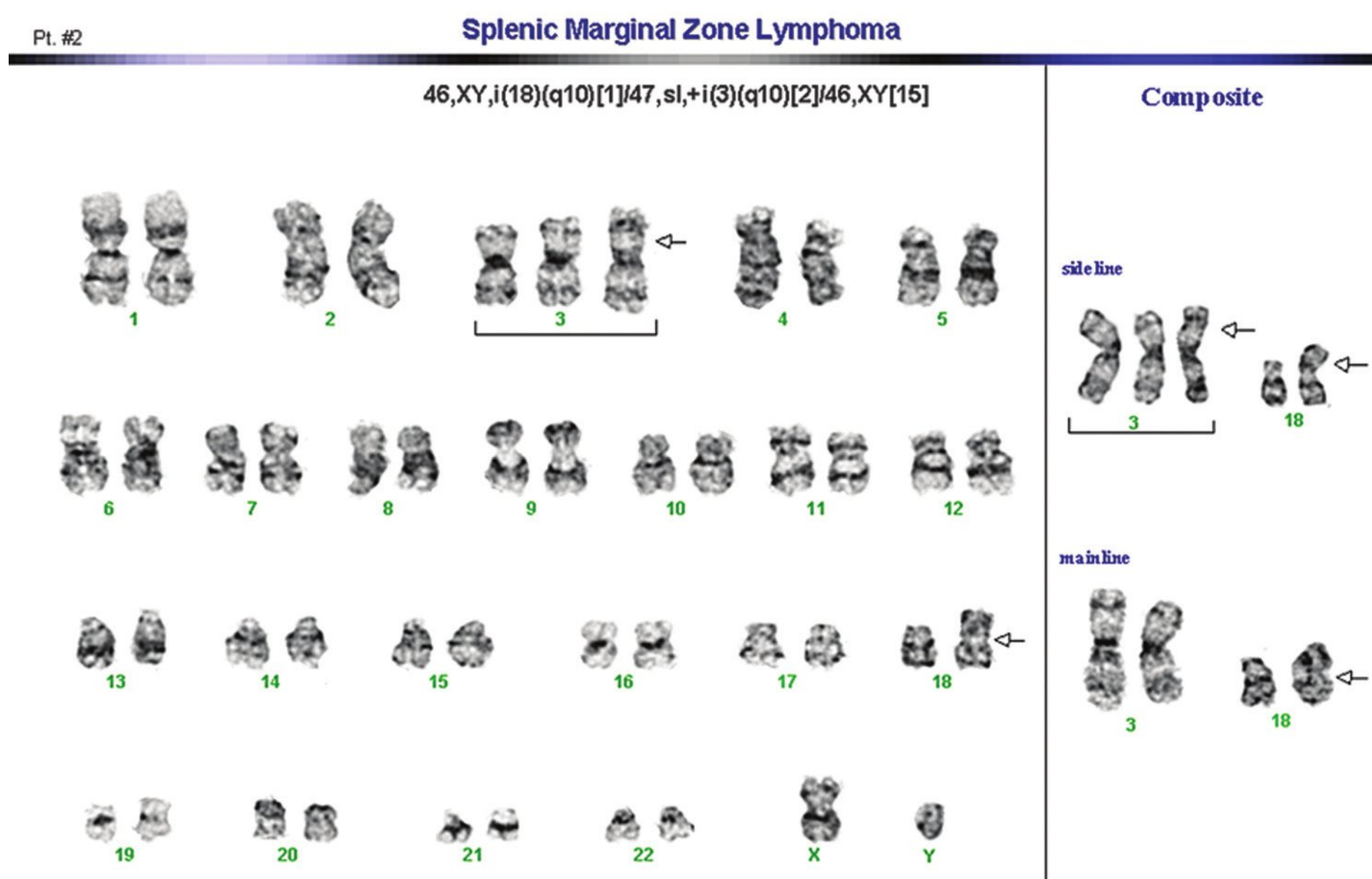


FIGURE 6.26.10 Karyotype from a splenectomy specimen demonstrates 46,XY,i(18)(q10)[1]/47,sl,+3(q10)[2]/46,XY[15]. Gain of chromosome 3 is a recurrent finding in SMZL. Gain of chromosome 18 is a recurrent finding in marginal zone lymphomas.

group showed that when patients received chemotherapy as first-line treatment or had a high leukocyte count ($>30,000/\text{mL}$) or lymphocytopenia ($<4,000/\text{mL}$), the prognosis was worse than it was in control groups (34). Other adverse factors include lymphocytosis ($>9,000/\text{mL}$), raised b_2 microglobulin, and the presence of a paraprotein or autoimmune phenomenon (31). In a study of 100 patients with SLVL, the 5-year overall survival was 78% (34).

In the current case, the immunophenotypes of the tumor cells from the peripheral blood, bone marrow, and spleen were comparable, showing a monoclonal IgM κ population with positive CD19, CD20, and HLA-DR, but no dual staining of CD19/CD5 was demonstrated. This immunophenotype can only help to exclude small lymphocytic lymphoma and mantle cell lymphoma. The diagnosis was made by examining the histologic features in the spleen. The woman showed symptoms of autoimmune hemolytic anemia, but lacked other adverse prognostic factors such as thrombocytopenia, leukocytosis, or lymphocytopenia. As a result, she had a complete remission after splenectomy and went back to work fulltime 1 year after splenectomy.

REFERENCES

1. Them H, Burger A, Keiditsch E, et al. Klinische Beobachtungen zur charakterisierung des splenomegalen Immunozytomas. *Med Klin*. 1977;72:1019–1032.
2. Spriano P, Barosi G, Invernizzi R, et al. Splenomegaly immunocytoma with circulating hairy cells. Report of eight cases and revision of the literature. *Haematologica*. 1986;71:25–33.
3. Neiman RS, Sullivan AL, Jaffe R. Malignant lymphoma simulating leukemic reticuloendotheliosis. A clinicopathologic study of ten cases. *Cancer*. 1979;43:329–342.
4. Palutke M, Tabaczka P, Mirchandani I, et al. Lymphocytic lymphoma simulating hairy cell leukemia. A consideration of reliable and unreliable diagnostic features. *Cancer*. 1981;48:2047–2055.
5. Fohlmiste I, Schaefer HE, Modder B, et al. Chronische lymphoproliferative Erkrankung unter dem Bild einer Haarzell-Leukemie. *Blut*. 1981;42:367–377.
6. Melo JV, Hedge V, Parreira A, et al. Splenic B-cell lymphoma with circulating villous lymphocytes. Differential diagnosis of B-cell leukemia with large spleens. *J Clin Pathol*. 1987;40:642–651.
7. Melo JV, Robinson DSF, Gregory C, et al. Splenic B-cell lymphoma with villous lymphocytes in the peripheral blood. A disorder distinct from hairy cell leukemia. *Leukemia*. 1987;1:294–299.
8. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of chronic (mature) B and T lymphoid leukemias. *J Clin Pathol*. 1989;42:567–584.
9. Schmid C, Kirkham N, Diss T, et al. Splenic marginal zone cell lymphoma. *Am J Surg Pathol*. 1992;16:455–466.
10. Isaacson PG, Matutes E, Burke M, et al. The histopathology of splenic lymphoma with villous lymphocytes. *Blood*. 1994;84:3828–3834.
11. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:2608–2101.
12. Isaacson PG, Piris MA, Castovsky D, et al. Splenic marginal zone lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:135–137.
13. Isaacson PG, Piris MA, Berger F, et al. Splenic B-cell marginal zone lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:185–187.
14. Catovsky D, Matutes E. Splenic lymphoma with circulating villous lymphocytes/splenic marginal-zone lymphoma. *Semin Hematol*. 1999;36:148–154.
15. Dunn-Walters D, Boursier L, Spencer J, et al. Analysis of immunoglobulin genes in splenic marginal zone lymphoma suggests ongoing mutation. *Hum Pathol*. 1998;29:585–593.
16. Pittaluga S, Verhoef G, Criel A, et al. “Small” B-cell non-Hodgkin’s lymphomas with splenomegaly at presentation are either mantle cell lymphoma or marginal zone cell lymphoma. *Am J Surg Pathol*. 1996;20:211–223.
17. Dufresne SD, Felgar RE, Sargent RL, et al. Defining the borders of splenic marginal zone lymphoma: a multiparameter study. *Hum Pathol*. 2010;41:540–551.
18. Sun T, Myron S, Brody J, et al. Splenic lymphoma with circulating villous lymphocytes. Report of seven cases and review of the literature. *Am J Hematol*. 1994;45:39–50.
19. Piris M, Foucar K, Mollejo M, et al. Splenic B-cell lymphoma/leukemia, unclassifiable. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:191–193.
20. Mollejo M, Camacho FI, Algara P, et al. Nodal and splenic marginal zone B cell lymphoma. *Hematol Oncol*. 2005;23:108–118.
21. Valensi F, Durand V, Bastenaire B, et al. Splenic B-cell lymphoma with villous lymphocytes (SLVL). A lymphocytic lymphoma simulating hairy cell leukemia. *Nouv Rev Fr Hematol*. 1990;32:409–414.
22. Kettle P, Morris TCM, Markey GM, et al. Tartrate resistant acid phosphatase positive splenic lymphoma. A relative benign condition occurring in a time-space cluster? *J Clin Pathol*. 1990;43:714–718.
23. Rousselet MC, Gardenbas-Pain M, Rainier CT, et al. Splenic lymphoma with circulating villous lymphocytes. Report of a case with immunologic and ultrastructural studies. *Am J Clin Pathol*. 1992;97:147–152.
24. Labouyrie E, Marit G, Vial JP, et al. Intrasinusoidal bone marrow involvement by splenic lymphoma with villous lymphocytes. A helpful immunohistologic feature. *Mod Pathol*. 1997;10:1015–1020.
25. Iannitto E, Ambrosetti A, Ammatuna E, et al. Splenic marginal zone lymphoma with or without villous lymphocytes. Hematologic findings and outcomes in a series of 57 patients. *Cancer*. 2004;101:2050–2057.
26. Franco V, Florena AM, Iannitto E. Splenic marginal zone lymphoma. *Blood*. 2003;101:2464–2472.
27. Oscier D, Owen R, Johnson S. Splenic marginal zone lymphoma. *Blood Rev*. 2005;19:39–51.
28. Boveri E, Arcaini L, Merli M, et al. Bone marrow histology in marginal zone B-cell lymphomas: correlation with clinical parameters and flow cytometry in 120 patients. *Ann Oncol*. 2009;20:129–136.
29. Petit B, Parrens M, Soubeyran I, et al. Among 157 marginal zone lymphomas, DBA.44 (CD76) expression is restricted to tumour cells infiltrating the red pulp of the spleen with a diffuse architectural pattern. *Histopathology*. 2009;54:626–631.
30. Melo JV, Robinson DSF, Catovsky D. The differential diagnosis between chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders. Morphological and immunological studies. In: Polliack A, Catovsky D, eds. *Chronic Lymphocytic Leukemia*. Chur, Switzerland: Harwood Academic Publishers; 1988:85–103.

31. Thieblemont C, Felman P, Callet-Bauchu E, et al. Splenic marginal zone lymphoma: a distinct clinical and pathological entity. *Lancet Oncol*. 2003;4:95–103.
32. Wu CD, Jackson C, Medeiros LJ. Splenic marginal zone cell lymphoma. An immunophenotypic and molecular study of five cases. *Am J Clin Pathol*. 1996;105:277–285.
33. Matutes E, Morilla R, Owusu-Audomah K, et al. The immunophenotype of splenic lymphoma with villous lymphocytes and its relevance to the differential diagnosis with other B-cell disorders. *Blood*. 1994;83:1558–1562.
34. Troussard X, Valensi F, Duchayne E, et al. Splenic lymphoma with villous lymphocytes. Clinical presentation, biology and prognostic factors in a series of 100 patients. *Br J Haematol*. 1996;91:731–736.
35. Kurtin PJ, Hobday KS, Ziesmer S, et al. Demonstration of distinct antigen profiles of small B-cell lymphomas by paraffin section immunohistochemistry. *Am J Clin Pathol*. 1999;112:319–329.
36. Baseggio L, Traverse-Glehen A, Petinataud F, et al. CD5 expression identifies a subset of splenic marginal zone lymphomas with higher lymphocytosis: a clinic-pathological, cytogenetic and molecular study of 24 cases. *Hematologica*. 2010;95:604–612.
37. Salido M, Baro C, Oscier D, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: a multicenter study of the splenic B-cell lymphoma group. *Blood*. 2010;116(9):1479–1488.
38. Berger F, Feldman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. *Blood*. 2000;95:1950–1956.
39. Ruiz-Ballesteros E, Mollejo M, Rodriguez A, et al. Splenic marginal zone lymphoma: proposal of new diagnostic and prognostic markers identified after tissue and cDNA microarray analysis. *Blood*. 2005;106:1831–1838.
40. Bahier DW, Pindzola JA, Swerdlow SH. Splenic marginal zone lymphomas appear to originate from different B cell types. *Am J Pathol*. 2002;161:81–88.
41. Algara P, Mateo MS, Sanchez-Beato M, et al. Analysis of the IgV(H) somatic mutations in splenic marginal zone lymphoma defines a group of unmutated cases with frequent 7q deletion and adverse clinical course. *Blood*. 2002;99:1299–1304.
42. Bates I, Bedu-Addo G, Ruthford TR, et al. Circulating villous lymphocytes—a link between hyperreactive malarial splenomegaly and splenic lymphoma. *Trans R Soc Trop Med Hyg*. 1997;91:171–174.
43. Hermine O, Lefrere F, Bronowicki JP, et al. Regression of splenic lymphoma with villous lymphocytes after treatment of hepatitis C virus infection. *N Engl J Med*. 2002;347:89–94.
44. Novera F, Arcaini L, Merli M, et al. High-resolution genome-wide array comparative genomic hybridization in splenic marginal zone B-cell lymphoma. *Hum Pathol*. 2009;40:1628–1637.
45. Troussard X, Mauvieux L, Radford-Weiss L, et al. Genetic analysis of splenic lymphoma with villous lymphocytes: a groupe Français d'Hématologie Cellulaire (GFHC) study. *Br J Haematol*. 1998;101:712–721.
46. Oscier DG, Matutes E, Gardiner A, et al. Cytogenetic studies in splenic lymphoma with villous lymphocytes. *Br J Haematol*. 1993;85:487–491.
47. Savilo E, Campo E, Mollejo M, et al. Absence of cyclin D1 protein expression in splenic marginal zone lymphoma. *Mod Pathol*. 1998;11:601–606.
48. Mulligan SP, Matutes E, Dearden C, et al. Splenic lymphoma with villous lymphocytes. Natural history and responses to therapy in 50 cases. *Br J Haematol*. 1991;78:206–209.

CASE 27

Extranodal Marginal Zone Lymphoma of Mucosa-Associated Lymphoid Tissue

CASE HISTORY

A 60-year-old man was admitted to the hospital because of dyspnea on exertion, shortness of breath, and pleuritic chest pain for 3 months. The patient also had a decreased appetite and an approximate 12-lb weight loss over a 6-month period prior to admission. Upon questioning, the patient admitted to having had fevers, chills, and occasional night sweats. Chest x-ray examination detected bilateral lung mass. The patient had undergone transbronchial biopsies and brushings, which were nondiagnostic. He was then referred to our hospital for a diagnosed wedge biopsy of his lung mass.

Physical examination on admission determined that his lungs were clear to auscultation bilaterally. There was no superficial lymphadenopathy, and his liver and spleen were not palpable. Hematologic workup showed a total leukocyte count of 5,200/mL, hematocrit 40%, and platelets 259,000/mL. His liver function tests, creatinine, and lactate dehydrogenase were within normal ranges. The chest

computed tomography (CT) scan revealed a 2 × 6 cm mass in the left lower lobe and a 2 × 3 cm mass in the right lower lobe. A video-assisted lung wedge biopsy was done, and a diagnosis of lymphoma was established.

The patient was treated with nine cycles of chlorambucil, and he continued to improve clinically. Successive CT scans revealed that his left lung lesion disappeared, but the right lung mass gradually increased in size. He also showed increasing fatigue and night sweats. A complete excision of the right lung lesion was performed 1 year after the first admission, and the patient was observed in the oncology clinic on a regular basis.

FLOW CYTOMETRY FINDINGS

Lung biopsy showed 34% CD2, 35% CD5, 1% CD10, 69% CD19, 0% CD19/CD5, 61% CD20, 10% CD23, 4% k, 56% l, and 2% CD14 (Fig. 6.27.1).

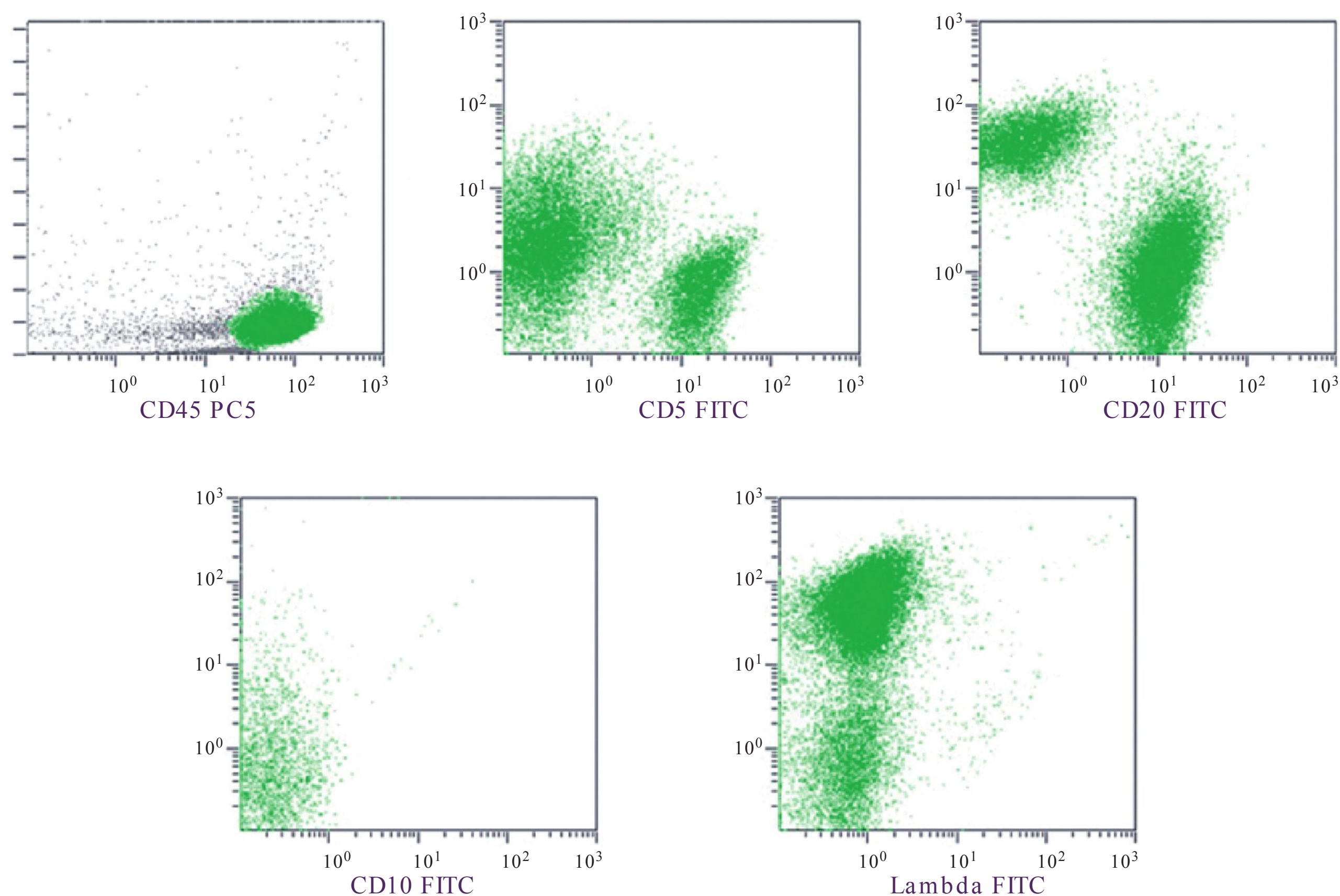


FIGURE 6.27.1 Flow cytometric histograms show positive reactions to CD19, CD20, and monoclonal k Ig. A T-cell component, as demonstrated by CD2 and CD5, is also present in the gated population. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

DISCUSSION

In 1983, Isaacson and Wright (1) developed the concept of lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma) for some low-grade lymphomas in the gastrointestinal (GI) tract. This concept was later extended to lymphomas of other extranodal sites (2). Since then, it has been found that these tumors are derived from the marginal zone B cells (3) and are related to monocytoid B-cell lymphoma (4,5). In the revised European–American Lymphoma and World Health Organization (WHO) classifications, monocytoid B-cell lymphoma and MALT lymphoma are designated nodal and extranodal marginal zone lymphoma, respectively (6–8). The relationship of splenic marginal zone lymphoma to these two tumors is still disputable (4).

In light of the current knowledge, it is apparent that most, if not all, of the MALT lymphomas develop after a specific antigenic stimulation, such as infection by *Helicobacter pylori* in gastric MALT lymphoma, or on an autoimmune background, such as those seen in the salivary glands of patients with Sjögren syndrome (myoepithelial sialadenitis) and in the thyroid gland of patients with Hashimoto thyroiditis (9). There are several other suspicious infectious agents associated with MALT lymphoma, but their etiologic role has not been established. These include *Borrelia burgdorferi* in cutaneous MALT lymphoma, *Campylobacter*

jejuni in intestinal MALT lymphoma, *Chlamydia psittaci* in ocular adnexal MALT lymphoma, and hepatitis C virus in nongastric MALT lymphoma (10,11). Furthermore, MALT lymphoma usually occurs in organs where lymphoid tissue is not present normally, such as the stomach, salivary gland, thyroid gland, and the bronchus. In these organs, the lymphoid tissue is called acquired MALT.

Morphology

MALT lymphoma is most commonly seen in the GI tract accounting for 50% of all cases (12). The stomach is the leading location, comprising 85% of the GI cases. Other relatively common sites include lung (14%), head and neck (14%), ocular adnexa (12%), skin (11%), thyroid (4%), and breast (4%) (13). On rare occasions, the central nervous system and the genitourinary tract can also be involved (14–16). MALT lymphoma is considered the most common primary lymphoma in the urinary bladder (16), the lungs (17), and the ocular adnexa (18).

Before the era of immunophenotyping and molecular genetics, MALT lymphoma in the above organs was probably misdiagnosed as pseudolymphoma. Because several criteria for lymphoid hyperplasia, such as polymorphous infiltration, intermixing plasma cells with a lymphoid population, and presence of germinal centers, overlap with the diagnostic features of MALT lymphoma, the confusion is understandable (19).

The major type of tumor cells may appear as centrocytes (small to medium-sized lymphocytes with cleaved nucleus and moderately abundant cytoplasm), monocytoid B cells (cells with abundant pale cytoplasm and a centrally located bean-shaped or round nucleus), or small lymphocytes. The tumor population is polymorphous with the presence of transformed blasts (centroblasts or immunoblasts) and plasma cells (9). When plasma cells are present, the existence of an intranuclear pseudoinclusion (the Dutcher body) is characteristic of this entity (Fig. 6.27.2). When blasts are >10% of the lymphoid population, it was defined as high-grade MALT lymphoma by some authors (20). However, only the low-grade lymphoma with predominance of small lymphocytes is included in the definition of MALT lymphoma by the WHO group (7,8). When a large number of blasts present in a low-grade MALT lymphoma, it is considered to be transformation of MALT lymphoma to a diffuse large-cell lymphoma rather than a high-grade MALT lymphoma (9).

The cellular component may vary from organ to organ. Centrocyte-like cells are predominant in the stomach, monocytoid B cells are commonly seen in the salivary glands, small lymphocytes are frequently present in the lung, and plasma and/or plasmacytoid cells are mainly seen in thyroid and skin (21).

These cells infiltrate around the germinal centers at the marginal zone with or without the separation by an attenuated mantle zone (9,19,22,23). The tumor cells expand from the germinal center to form a dense infiltrate, causing effacement of the normal architecture (Fig. 6.27.3). One of the characteristic features of MALT lymphoma is the invasion of epithelial structures to form lymphoepithelial lesions (Figs. 6.27.4 and 6.27.5). The tumor cells may also invade the germinal center, which is referred to as follicular colonization (Fig. 6.27.6). The demonstration of the lymphoepithelial lesion can be facilitated by the use of cytokeratin (Fig. 6.27.7)

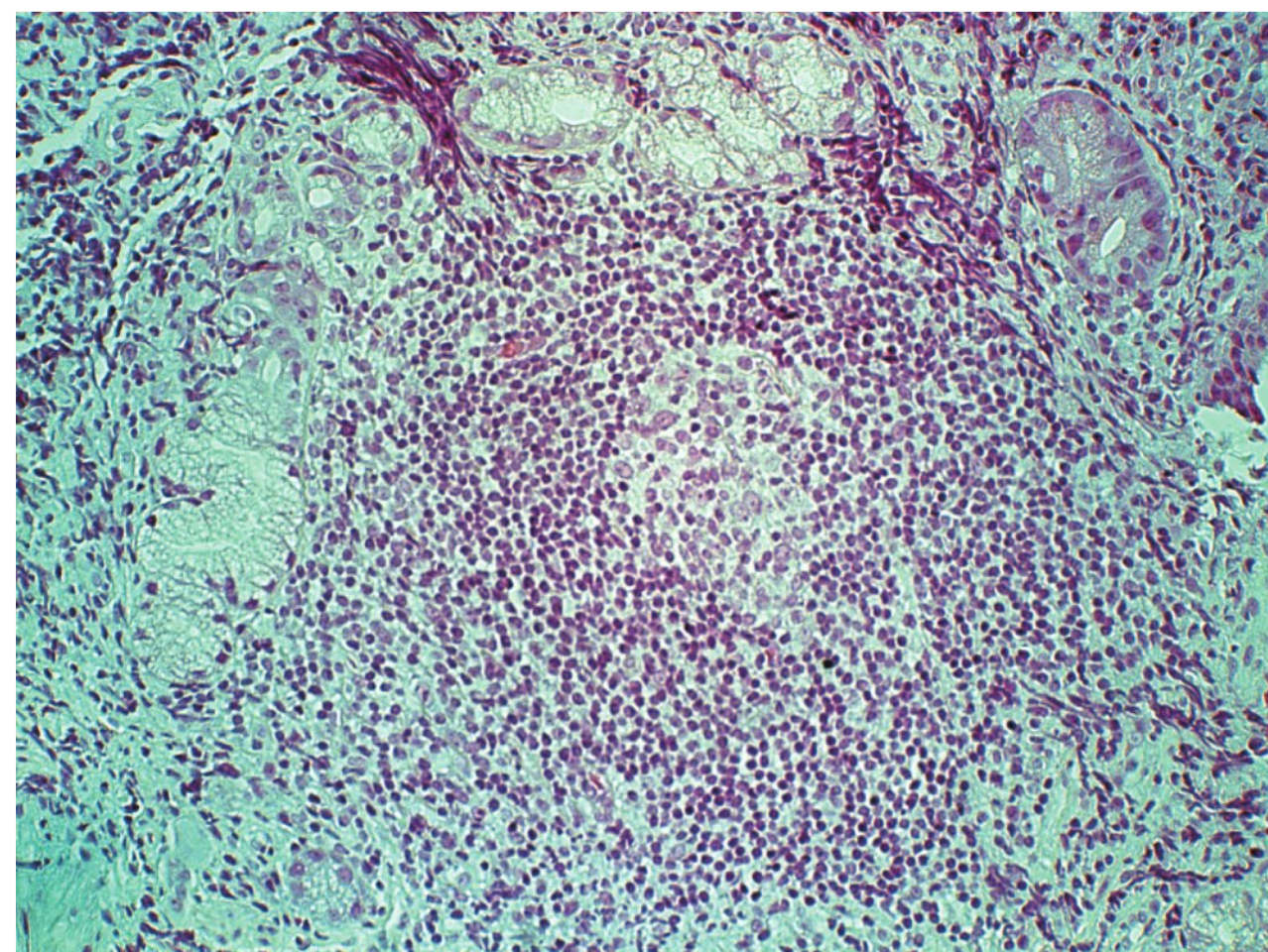


FIGURE 6.27.3 A germinal center is surrounded by a broad mantle zone of small lymphocytes, infiltrating the adjacent gastric mucosa in a case of lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma). Hematoxylin and eosin, 20× magnification.

and B-cell (Fig. 6.27.8) stains, whereas the detection of follicular colonization can be facilitated by the use of CD21 or CD35 to show the follicular dendritic meshwork (12,21).

However, the pathologic features may vary from site to site (21). In the stomach, lymphoepithelial lesions are considered essential to the diagnosis of MALT lymphoma. In the lungs, it is characterized by the presence of a nodular pattern with lymphangitic tracking at the periphery (Fig. 6.27.9). Lymphoepithelial lesions can be seen in the bronchus (Fig. 6.27.10). In the salivary gland, the monocytoid B cells form broad interconnecting strands that surround and invade epimyoepithelial islands and displace the germinal center (Fig. 6.27.11) (21,22). The thyroid

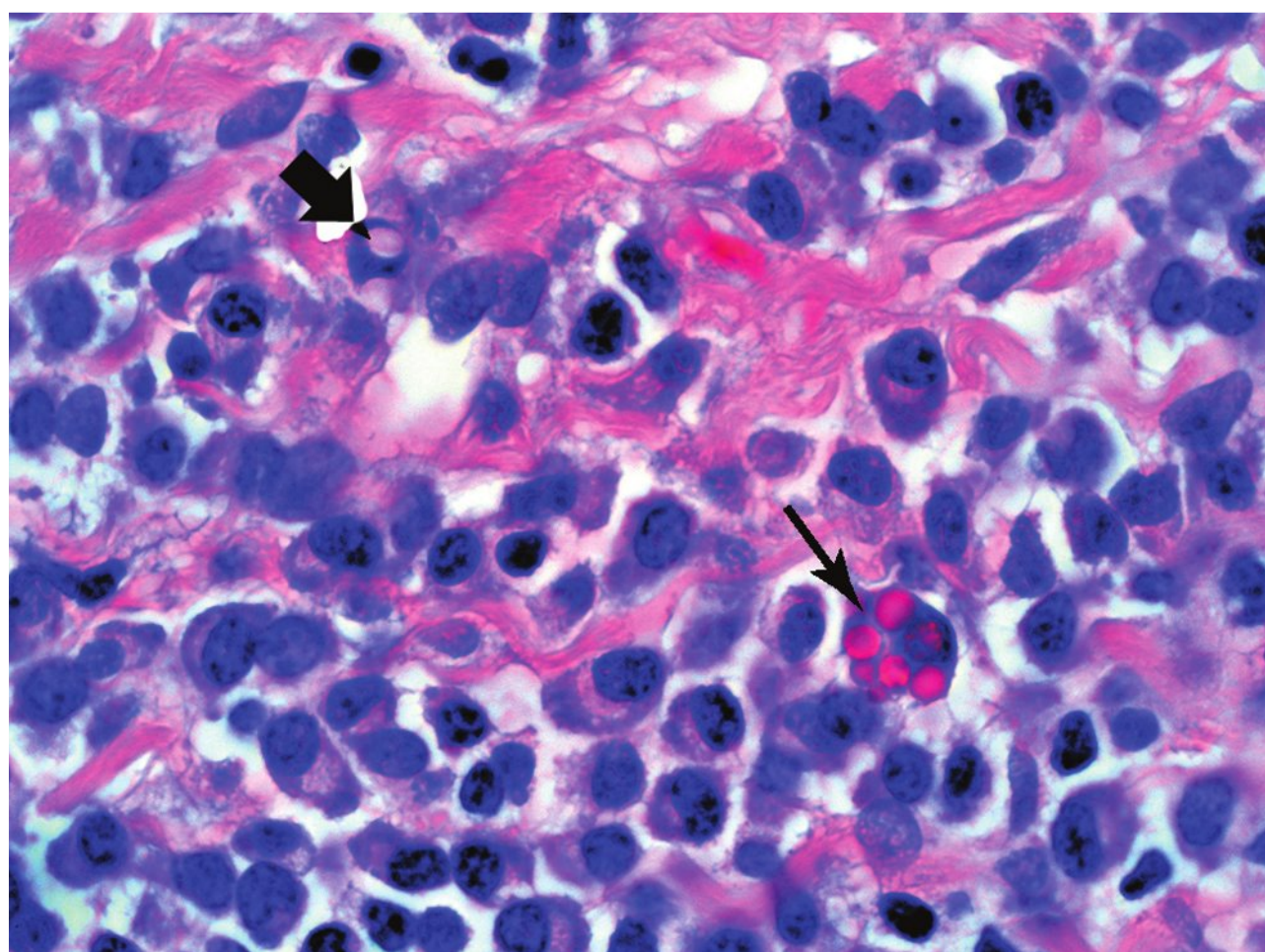


FIGURE 6.27.2 Intranuclear Dutcher body (broad arrow) and intracytoplasmic Russell bodies (narrow arrow) are demonstrated among a cluster of lymphoplasmacytic cells in a case of lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma). Hematoxylin and eosin, 100× magnification.

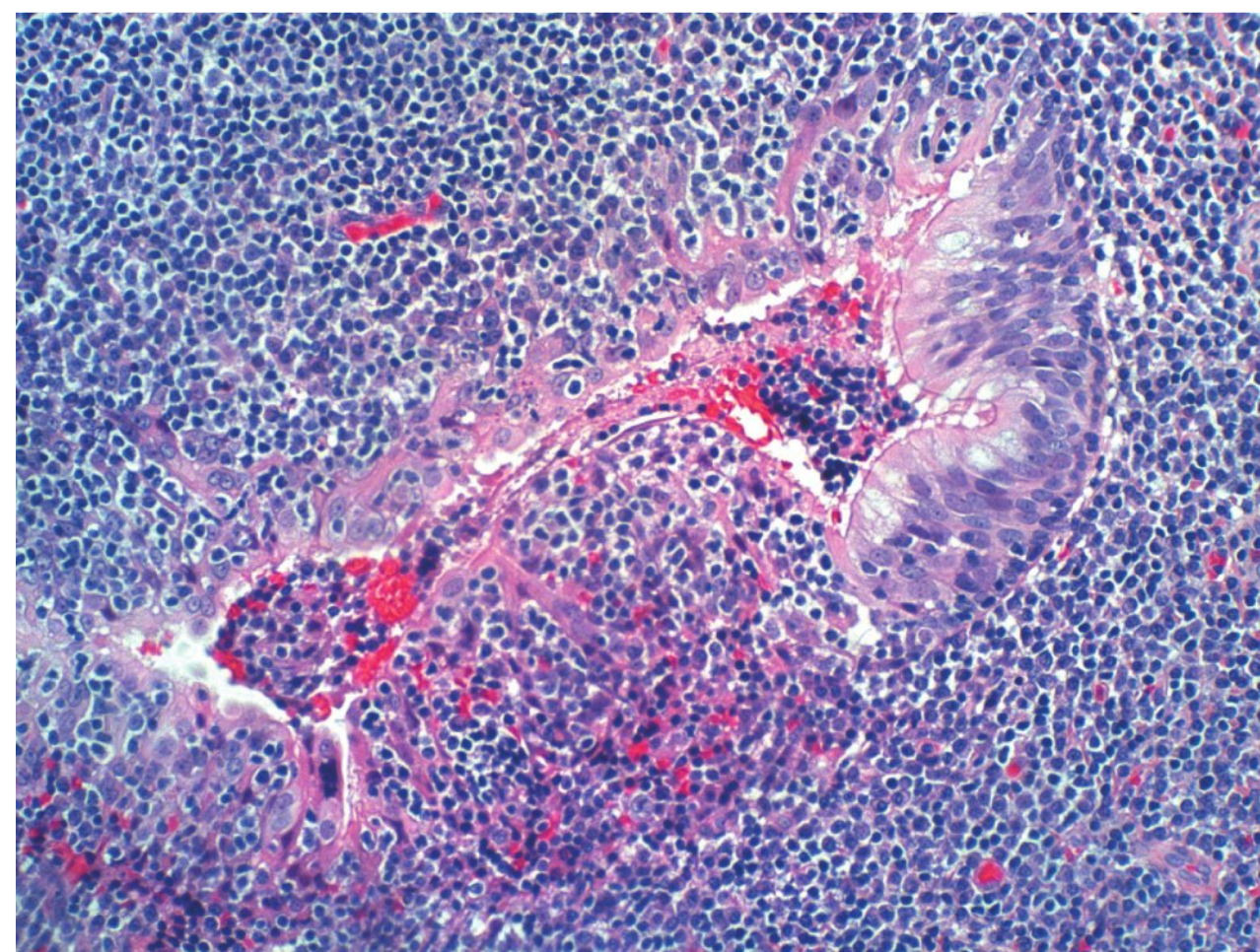


FIGURE 6.27.4 Prominent lymphoepithelial lesion shows a large gland being destroyed by small tumor cells. Hematoxylin and eosin, 20× magnification.

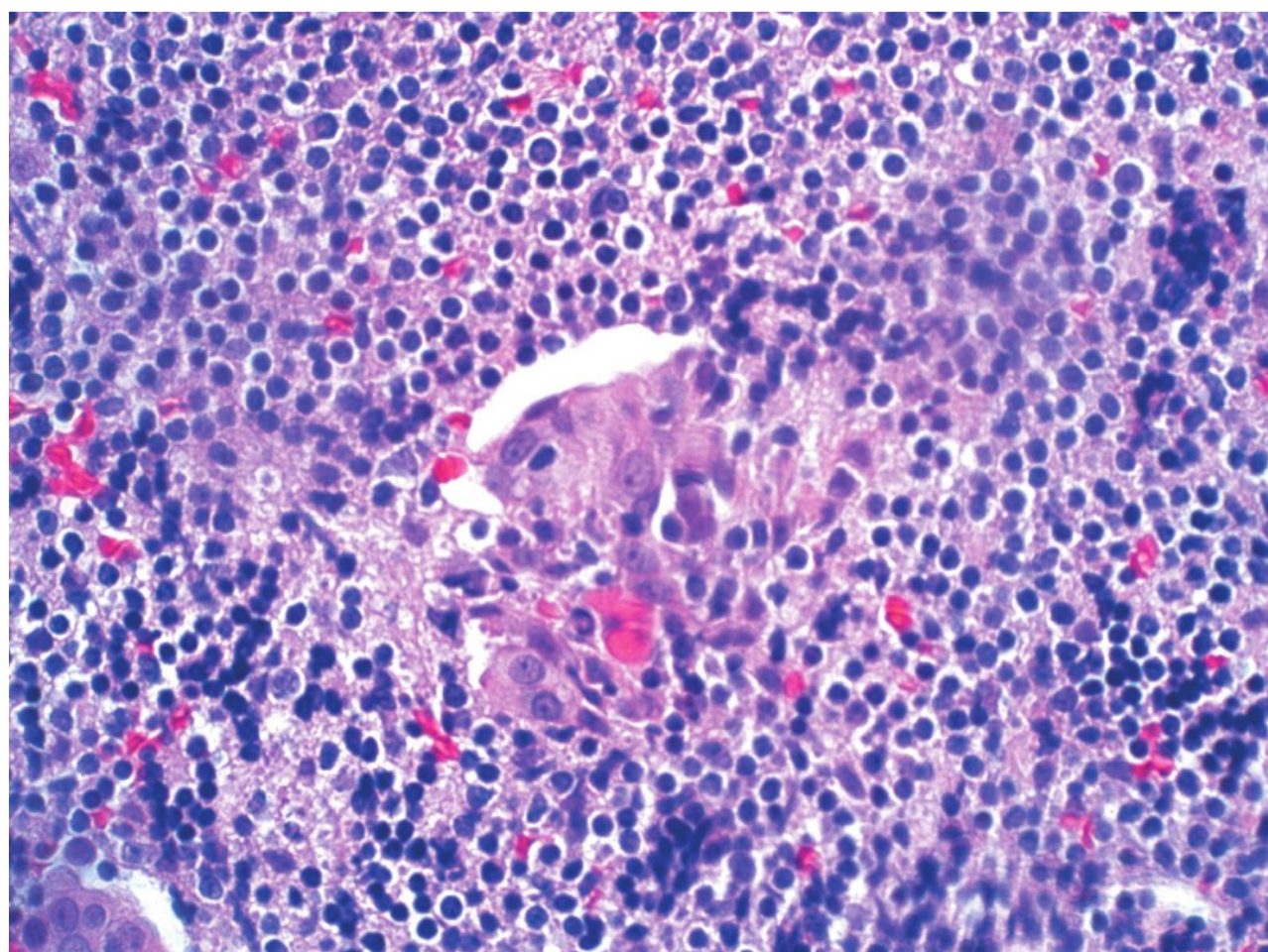


FIGURE 6.27.5 Residual epithelial fragments are surrounded by centrocyte-like cells in a case of lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma). Hematoxylin and eosin, 40× magnification.

gland may show extensive lymphoid infiltrates around residual germinal centers, effacing the normal architecture. Plasmacytic differentiation is a constant feature (8). Follicular colonization and lymphoepithelial lesions may be present but are not essential for the diagnosis.

The ocular MALT lymphoma shares the common feature of lymphoepithelial lesions and follicular colonization (18). Cutaneous MALT lymphoma is characterized by lymphoplasmacytic infiltration in the upper dermis with lymphoepithelial lesions involving hair follicles, eccrine sweat glands, and sebaceous glands (21,23,24). Mammary MALT lymphoma shows no specific features (21,25). The cellular components are mainly monocytoid B cells with admixed plasma cells surrounding germinal centers. Lymphoepithelial lesions and follicular colonization may be present.

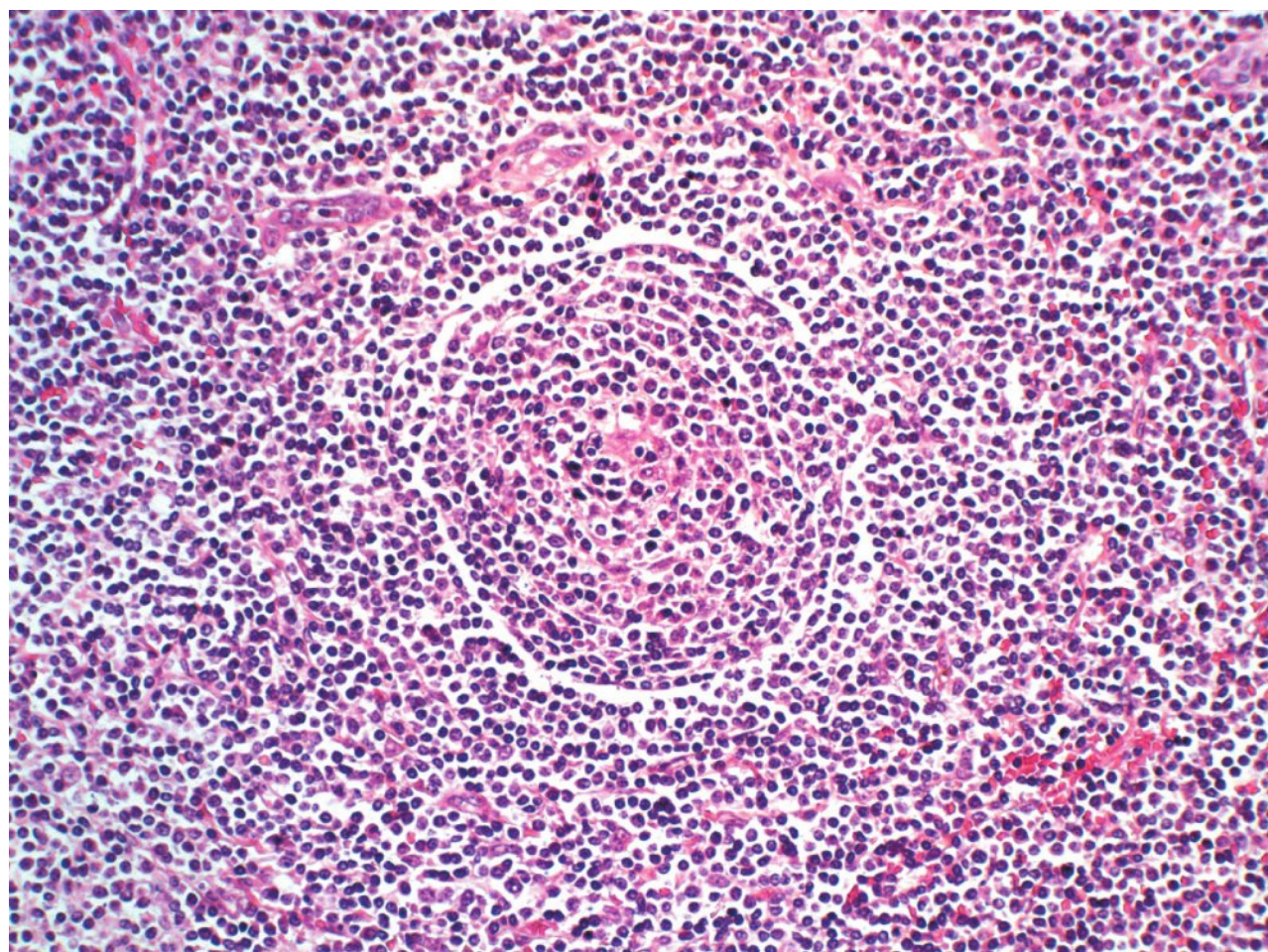


FIGURE 6.27.6 A colonized lymph follicle is surrounded by numerous small tumor cells. Hematoxylin and eosin, 20× magnification.

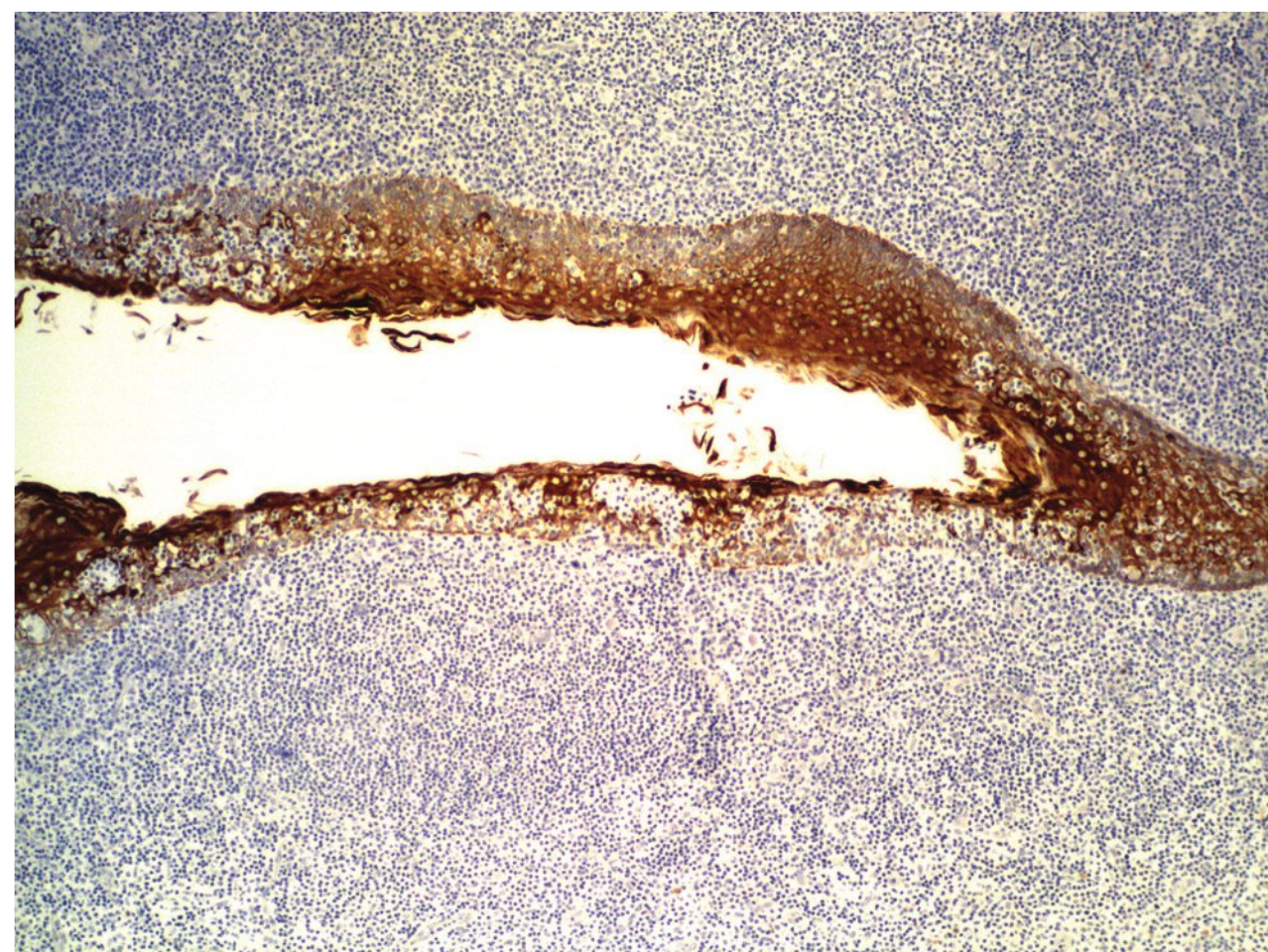


FIGURE 6.27.7 Cytokeratin stain demonstrates the invasion of tumor cells to the epithelium (lymphoepithelial lesion). Immunoperoxidase, 10× magnification.

A special form of MALT lymphoma in the small intestine is associated with a-chain disease, which is now designated immunoproliferative small intestinal disease (IPSID) (26,27). This subtype of MALT lymphoma is histologically similar to other subtypes, but presents with striking plasmacytic differentiation.

Several non-Hodgkin lymphomas may mimic MALT lymphoma. When follicular colonization is prominent, the histologic features may be similar to those of follicular lymphoma. However, the residual germinal centers show a polyclonal immunoglobulin (Ig) pattern and are not reactive to bcl-2, whereas follicular lymphoma shows no expansion of marginal zone and is positive for bcl-2 with a monoclonal Ig pattern (28). When lymphoplasmacytic infiltration is prominent, the differential diagnosis is lymphoplasmacytic

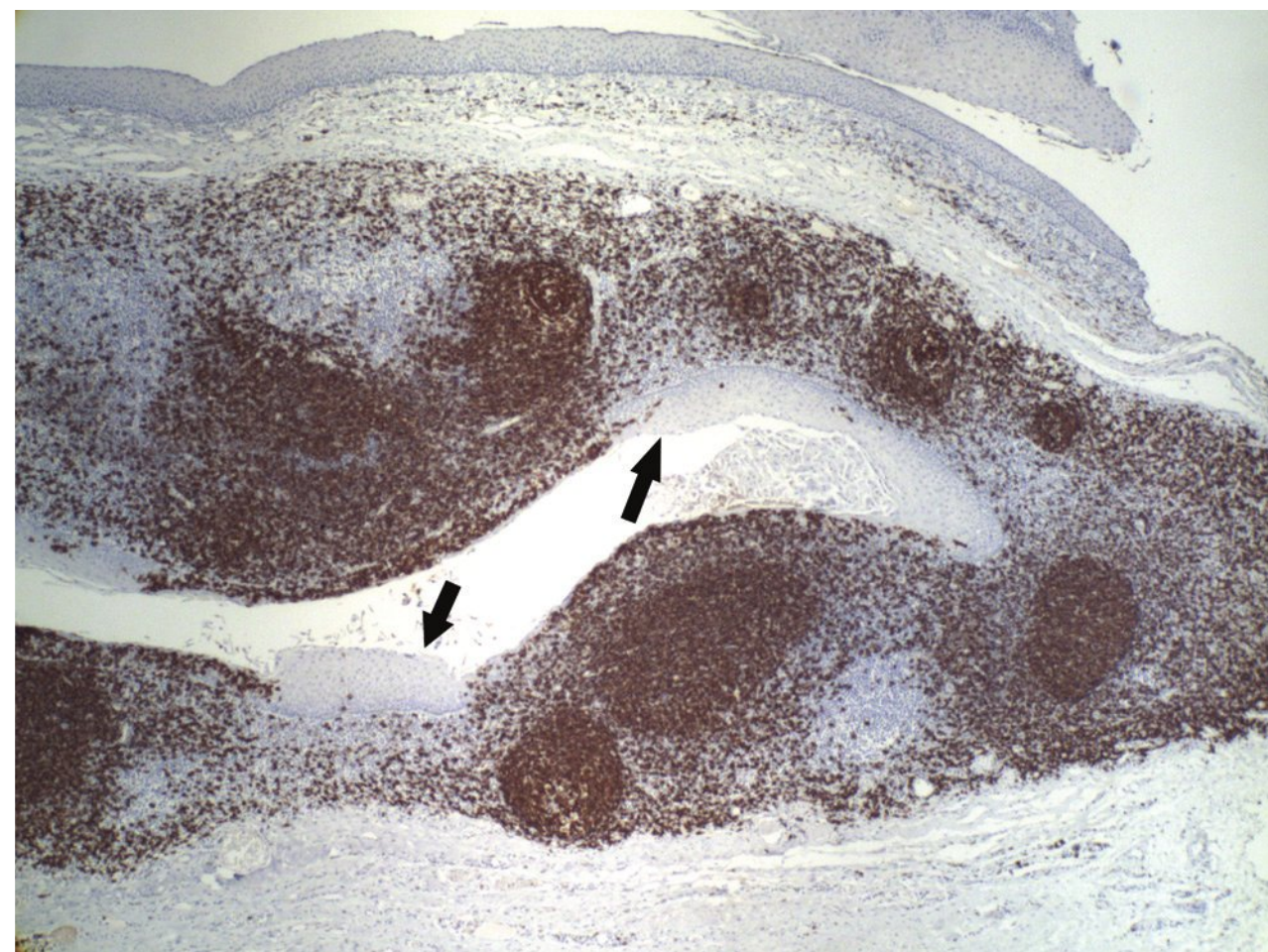


FIGURE 6.27.8 CD20 stain demonstrates enlarged lymphoid follicles, expansion of marginal zone and infiltration as well as destruction of adjacent epithelium. Residual epithelium is visible in some areas (arrows). Immunoperoxidase, 10× magnification.

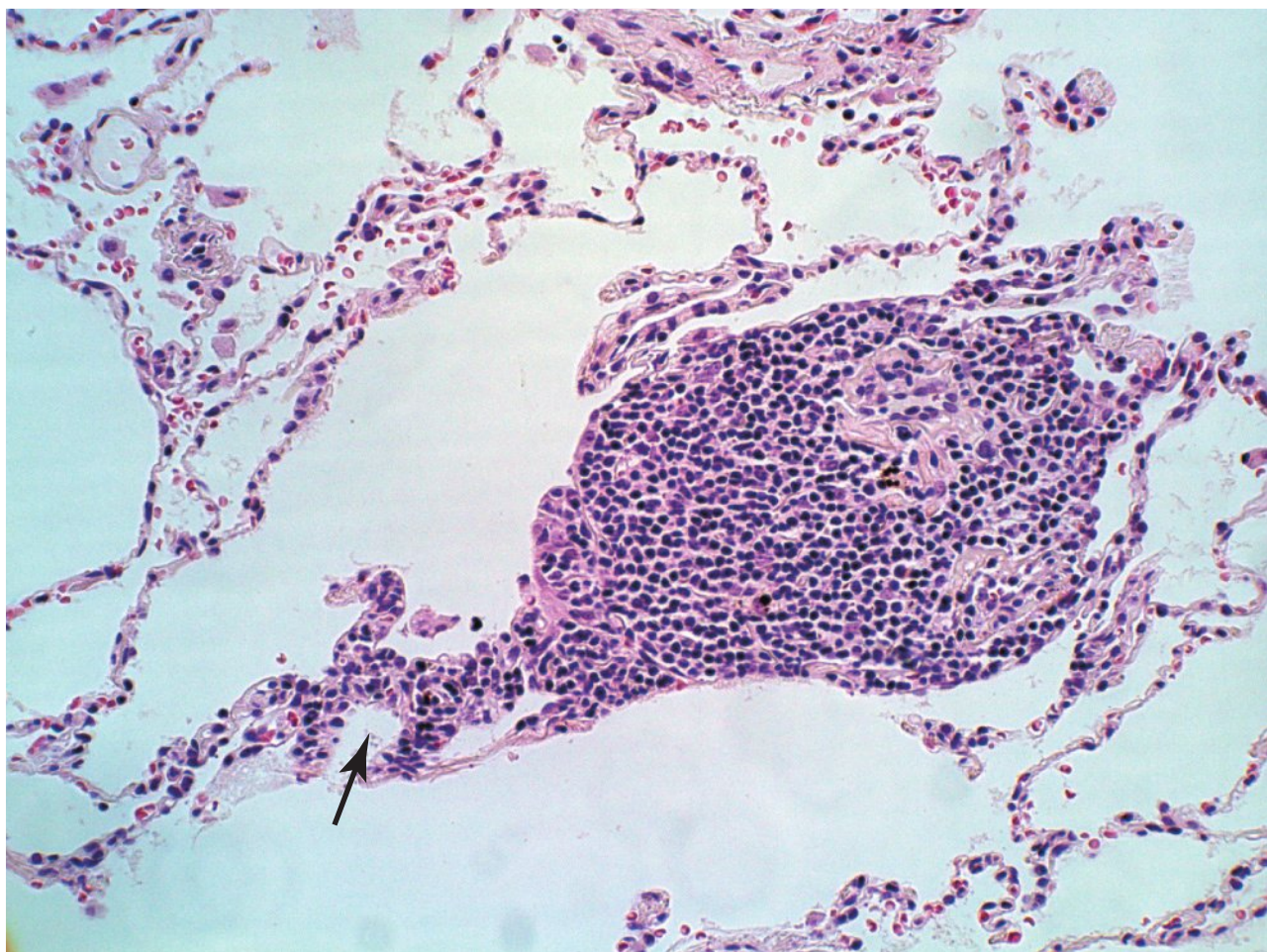


FIGURE 6.27.9 Pulmonary lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma) case shows nodular tumor cell infiltration with lymphangitic tracking in the alveolar septum (arrow). Hematoxylin and eosin, 20× magnification.

lymphoma/immunocytoma. However, if the tumor is located in an extranodal location, a diagnosis of MALT lymphoma is preferred (22). Immunocytoma also shows a monomorphous appearance and absence of germinal centers (21). Mantle cell lymphoma usually shows a monomorphous infiltration without the presence of plasma cells and transformed large cells. Small lymphocytic lymphoma may mimic MALT lymphoma with complete effacement of the normal architecture. The presence of proliferation center and the immunophenotype can help to establish the diagnosis of the former. The characteristic morphologic features in MALT lymphoma are summarized in Table 6.27.1.

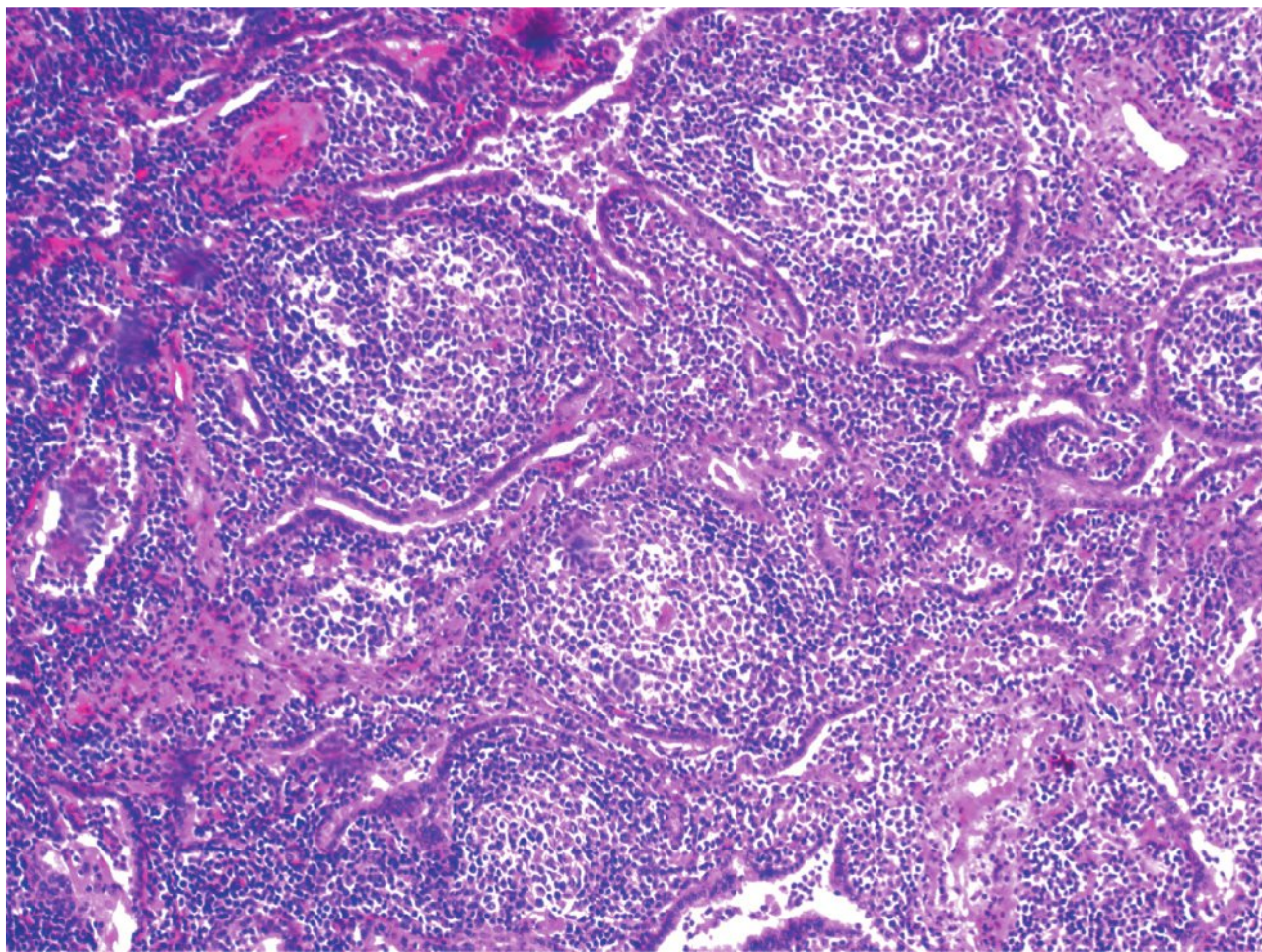


FIGURE 6.27.10 Pulmonary case of lymphoma of the mucosa-associated lymphoid tissue (MALT lymphoma) reveals multiple lymphoid follicles with prominent germinal centers, replacing the normal parenchyma and compressing the bronchioles. Hematoxylin and eosin, 10× magnification.

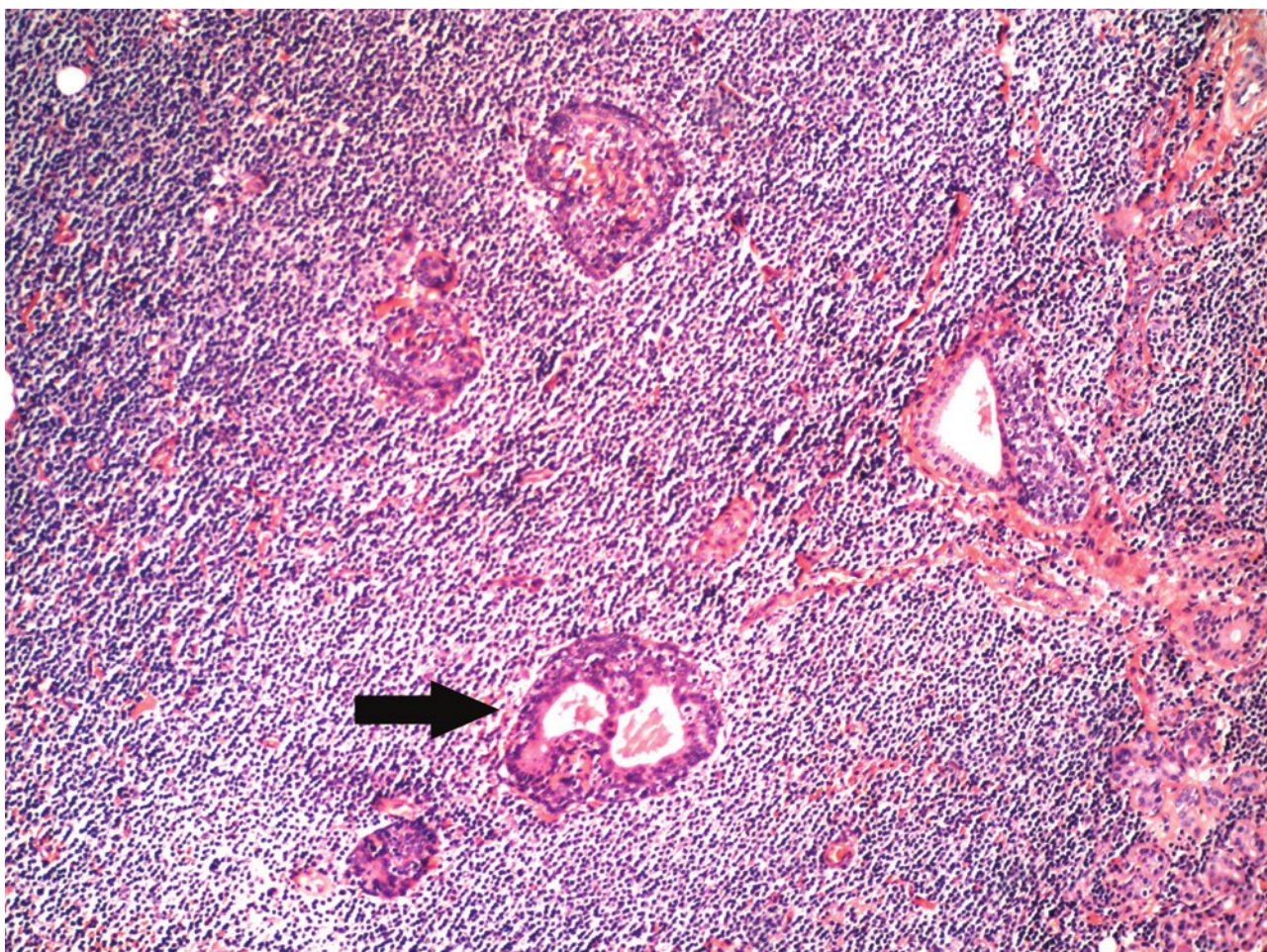


FIGURE 6.27.11 Biopsy of salivary gland shows three epimyoeptelial islands and one salivary duct with condensation of the epithelium and narrowing of the lumen (arrow). Hematoxylin and eosin, 10× magnification.

Immunophenotype

There are no specific markers for MALT lymphoma. The role of immunophenotyping is to demonstrate a monoclonal B-cell population (CD19+, CD20+, CD22+, CD79a+, bcl-2±) with the absence of specific markers for other non-Hodgkin lymphomas (12,22,29,30). For instance, the absence of CD5 and CD23 excludes the diagnosis of small lymphocytic lymphoma and chronic lymphocytic leukemia. The absence of CD5 and bcl-1 and/or cyclin D1 rules out mantle cell lymphoma. Negative reactions to CD10 and bcl-6 in the germinal centers are against the diagnosis of follicular lymphoma (31). The surface Ig on the tumor cells is most frequently IgM, but in the case of a α -chain disease, only a Ig is present without the light-chain expression.

TABLE 6.27.1	
Characteristic Morphologic Features of MALT Lymphoma	
Histologic pattern	Lymphoepithelial lesions, marginal zone infiltration, and follicular colonization
Cytology	Polymorphic population with mainly monocytoid B cells, centrocyte-like cells, or small lymphocytes Plasma cells with Dutcher bodies and a few large blasts may be present.
Specific features	Combination of histologic pattern and cytology

MALT, mucosa-associated lymphoid tissue.

MALT lymphoma is difficult to distinguish from nodal and splenic marginal zone B-cell lymphomas by immunophenotyping, but IgD is positive in splenic marginal zone lymphoma and some cases of nodal marginal zone B-cell lymphoma, whereas it is consistently negative in MALT lymphoma (32). The reaction to CD43 is variable, and CD23 and CD5 can be positive in a few cases of MALT lymphoma (1,33). However, when CD5 is positive, the diagnosis of MALT lymphoma should be made only with definitive support by other parameters. In the few reported cases of CD5-positive MALT lymphoma, a tendency of recurrence, dissemination, and leukemic manifestation was shown (33). Nevertheless, cases of CD5-positive MALT lymphoma with an indolent clinical course have also been reported (31).

As mentioned before, the demonstration of a follicular meshwork by CD21 or CD35 is helpful in recognizing follicular colonization, and the demonstration of the epithelial component by cytokeratin stain is useful in detection of the lymphoepithelial lesions. As a low-grade lymphoma, MALT lymphoma usually shows a low proliferation fraction as expressed by Ki-67 staining, which is in marked contrast to the residual germinal centers that show a high proliferation fraction (28). DNA analysis also displays low S-phase fractions ($\leq 3\%$) in MALT lymphoma, consistent with the above finding (29).

The current case showed the characteristic pulmonary symptoms with multiple lesions in the lung. The lung biopsy revealed a multinodular pattern and lymphangitic spreading of the tumor cells. The bronchial epithelium was extensively infiltrated by the tumor cells with prominent destruction and replacement of the normal architecture. The flow cytometric findings of negative reactions to CD5/CD19, CD10, and CD23 are helpful in distinguishing it from other low-grade small-cell lymphomas. A single lung lesion can be treated effectively with surgical excision, but the patient had bilateral lesions and was initially treated with chemotherapy until the left lung lesion disappeared. The patient had an uneventful recovery after excision of the right lung lesion and was in a long-term remission.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometric analysis may help to exclude other lymphomas by the negative reactions to CD5, CD10, and CD23. In CD10-negative follicular lymphoma, the low mean channel fluorescence for CD19 helps to distinguish it from MALT

lymphoma, which shows a high intensity of CD19 (34). The strong expression of CD22 combined with expression of CD25 help to distinguish lymphoplasmacytic lymphoma from MALT lymphoma (34). Immunohistochemistry, in addition, can also help to detect follicular colonization by the demonstration of the follicular dendritic meshwork and lymphoepithelial lesion by the demonstration of the cytokeratin-positive epithelial cells. Recent findings of the presence of gene products in the tumor cells may provide some positive markers for the diagnosis of MALT lymphoma (10). In cases with t(11;18) and t(1;14), nuclear bcl-10 can be demonstrated in the tumor cells. In contrast, cytoplasmic bcl-10 is shown in cases with t(14;18). In addition, cytoplasmic malt1 protein is present in cases with t(14;18) and nuclear foxp1 protein in cases with t(3;14).

Molecular Genetics

MALT lymphoma shows somatic hypermutation of Ig heavy-chain genes and extensive ongoing somatic mutation that put it into the late memory B-cell stage, presumably due to interaction with the follicular center and the effect of antigenic stimulation (9,35).

Because the distinction in morphology between MALT lymphoma and lymphoid hyperplasia is sometimes blurred, molecular genetic techniques are helpful for the differential diagnosis. The identification of Ig gene rearrangements by polymerase chain reaction in frozen or paraffin sections is particularly useful in establishing the diagnosis of MALT lymphoma (36). However, Ig gene rearrangements can be demonstrated in uncomplicated gastritis, and false-negative results are sometimes encountered in confirmed lymphoma cases (9,37). Therefore, the interpretation of polymerase chain reaction results should be in conjunction with morphology and immunophenotyping. Nevertheless, a study showed that, even a lesion with a low score according to the Wotherspoon–Isaacson histologic scoring system, if monoclonality is demonstrated, the lesion may relapse in further follow-up (36).

There have been extensive molecular genetic studies in MALT lymphoma (Table 6.27.2) (9,38,39). The accumulated data demonstrate a close correlation between the karyotypes and clinical manifestations, including the site of the lymphoma, therapeutic response, and the potential for high-grade transformation.

TABLE 6.27.2

Characteristics of Molecular Genetic Findings in MALT Lymphoma

Translocation	Genes involved	Additional aberrations	Anatomic sites
t(11;18)(q21;q21)	API2-MALT1	None	GI tract, lung, head, and neck
t(1;14)(p22;q32)	BCL10-IgH	None	Lung, stomach, skin
t(1;2)(p22;p12)	BCL10-IgL _k	None	Lung, stomach, skin
t(14;18)(q32;q21)	IgH-MALT1	Trisomy 3, 12, or 18	Lung, liver, ocular adnexa, skin
t(3;14)(p14;q32)	FOXP1-IgH	Trisomy 3	Thyroid, ocular adnexa, skin

MALT, mucosa-associated lymphoid tissue; GI, gastrointestinal; Ig, immunoglobulin.



t(11;18)(q21;q21)

This translocation is the most common chromosomal aberration demonstrated in MALT lymphoma, with a frequency varying from 13.5% to 35% (10,39). It has been mostly seen in MALT lymphoma of the GI tract and lung, but also has been reported in the head and neck. Molecular characterization has demonstrated the inhibitor of apoptosis 2 (API2) gene on 11q21 and the MALT lymphoma-associated translocation (MALT1) gene on chromosome 18q21. As a result of the translocation, an API2-MALT1 fusion gene is formed, which encodes for the api2-malt1 protein. This fusion gene and protein lead to increased inhibition of apoptosis with a resultant survival advantage of the tumor cells, independent of antigen (39). The api2-malt1 fusion protein may also activate the nuclear factor-kB (NF-kB) pathway, which is considered the unifying mechanism for several cytogenetic aberrations (10). NF-kB appears to drive antigen-independent growth of the lymphoma cells so that eradication of *H. pylori* or other antigens will no longer be effective in the treatment of the patients (40).

Cases with t(11;18) are unresponsive to *H. pylori* eradication therapy, partly because of the autonomous growth of the tumor cells and partly because most such cases are *H. pylori* negative (40). Another characteristic feature of t(11;18) is the association of nuclear bcl-10 protein expression, in contrast to the normal expression of cytoplasmic bcl-10 protein in the germinal center cells (40). Weak cytoplasmic malt1 protein expression is also present (38).

Clinically, cases with t(11;18) usually present with advanced-stage disease, but are unlikely to develop secondary chromosomal abnormalities or to transform into diffuse large-cell lymphoma (10,35). t(11;18) is highly specific for MALT lymphoma, as it has not been reported in nodal or splenic marginal zone lymphomas or other B-cell neoplasms (31).

t(1;14)(p22;q32) or t(1;2)(p22;p12)

This translocation occurs in 1% to 2% of MALT lymphoma and has been reported in cases involving the lung, stomach, and skin (10). As a result of the translocation, the BCL10 gene on chromosome 1p22 is relocated to chromosome 14, under the control of the IgH enhancer region, or relocated to chromosome 2, under the control of the IgL_k region. As a result, bcl-10 protein is overexpressed in the nuclei of the tumor cells. The bcl-10 protein, in turn, activates the NF-kB pathway. A weak cytoplasmic malt1 staining is also present in tumors with this karyotype (38).

t(1;14) and t(1;2) are specific for MALT lymphoma and have not been reported in other lymphomas. Because these aberrant karyotypes activate the same pathway as t(11;18), patients also present with advanced-stage disease and are unlikely to respond to *H. pylori* eradication (41).

t(14;18)(q32;q21)

This karyotypic abnormality occurs in 15% to 20% of MALT lymphoma, mainly involving the non-GI MALT lymphomas, such as the liver, lung, ocular adnexa, and skin (10,39). As a result of the translocation, the MALT1 gene on chromosome 18q21 is juxtaposed with the IgH enhancer region, resulting in MALT1 overexpression. This overexpression

leads to the activation of the NF-kB pathway. Bcl-10 protein is also expressed in the cytoplasm of the tumor cells. Cases with t(14;18) frequently harbor additional karyotypic abnormalities, including trisomies 3 and/or 12 and 18 (10).

t(3;14)(p14;q32)

This translocation involves the forkhead box protein P1 (FOXP1) genes on chromosome 3p14 relocating to chromosome 14 under the control of the IgH enhancer. As a result, foxp1 protein is overexpressed, but it is unclear how this overexpression induces tumorigenesis (39). The overexpression of foxp1 protein has also been found in a subset of diffuse large B-cell lymphoma, and this finding raises the possibility that MALT lymphoma with this translocation may be at risk to transform to diffuse large B-cell lymphoma (10). This aberration has been encountered in 10% of MALT lymphoma cases, and is present in the thyroid, ocular adnexa, and skin (10,39). Most t(3;14)-positive cases also harbor additional genetic abnormalities, such as trisomy 3 (39).

The mechanism underlying the transformation from low-grade MALT lymphoma to a high-grade lymphoma is still unclear. However, a number of genetic aberrations have been present in cases with histologic transformation, such as p53 allelic loss and mutation, hypermethylation of p15 and p16, and p16 deletions (10,42).

Although bcl-2 antigen may be present in MALT lymphoma, rearrangement of BCL-2 gene has not been demonstrated in this tumor. BCL-1 and c-MYC rearrangement are also not present (42,43), but c-MYC and p53 mutations are encountered in MALT lymphoma (42–44). In the earlier literature, BCL-6 rearrangement and mutation were demonstrated only in high-grade MALT lymphoma (44,45). However, a recent study with the fluorescence in situ hybridization technique confirmed BCL-6 translocation in 6 of 306 cases of MALT lymphoma, involving the stomach, salivary gland, lung, skin, and thyroid (46). The salient features for laboratory diagnosis of MALT lymphoma are summarized in Table 6.27.3.

TABLE 6.27.3

Salient Features for Laboratory Diagnosis of MALT Lymphoma

1. Monoclonal B-cell population, positive for the following B-cell markers: CD19, CD20, CD22, CD79a, bcl-2
2. Absence of the following markers: CD5, CD10, CD23, bcl-1/cyclin D1, and IgD
3. Immunohistochemical stain may demonstrate bcl-10 and malt1 protein in cases with special karyotypes.
4. Rearrangement of Ig genes
5. No rearrangement of BCL-1, BCL-2, and c-MYC, but rearrangement of BCL-6 can be seen in a subset of MALT lymphoma.
6. Demonstration of t(11;18), t(1;14), or t(1;2) is diagnostic for MALT lymphoma.

Ig, immunoglobulin; MALT, mucosa-associated lymphoid tissue.

Clinical Manifestations

Low-grade gastric and pulmonary MALT lymphomas are usually seen in individuals older than 50 years, but younger patients have been encountered more frequently in recent years (9). For instance, MALT lymphoma has been reported in pediatric cases with and without human immunodeficiency virus infection (47,48).

As mentioned before, one of the unique features of MALT lymphoma is the demonstration of antigenic stimulation as the mechanism of tumorigenesis. The most striking example is *H. pylori* infection in gastric MALT lymphoma. It is hypothesized that *H. pylori* infection may stimulate both the neoplastic B cells and the *H. pylori*-specific tumor-infiltrating T cells (49). These T cells provide the microenvironment for tumor cell proliferation. This theory is supported by the experiment that the cultured tumor cells maintained in standard conditions die in 5 days without the coexistence of *H. pylori* and T lymphocytes (9). Therefore, even if the tumor cells spread to distant sites, they would fail to grow there in the absence of *H. pylori* and the T cells activated by the organism.

The most important evidence to support the etiologic role of *H. pylori* in MALT lymphoma is that the eradication of this organism results in regression of the tumor in 50% to 100% of cases in various series (9,31,50). However, as mentioned before, cases with t(11;18) are significantly less likely to respond to *H. pylori* eradication therapy (40).

The clinical symptom depends on the involved organ. For instance, patients with gastric MALT lymphoma may have nonspecific dyspepsia and epigastric pain (40), and those with pulmonary MALT lymphoma may have cough, dyspnea, chest pain, and hemoptysis (51). The symptoms of MALT lymphoma in the ocular adnexa depend on the location. Its most frequent site of involvement is the orbit (□40%), followed by conjunctiva (35% to 40%), lacrimal gland (10% to 15%), and eyelid (□10%) (52). Monoclonal gammopathy is present in about one third of patients with MALT lymphoma in two studies, but its clinical relevance is controversial (53,54). For unknown reason, paraprotein is especially commonly seen in patients with pulmonary MALT lymphoma (17,55).

Most patients have an indolent clinical course with localized symptoms. However, a recent study showed that dissemination of MALT lymphoma occurred in 54 of 158 patients, but the 5- and 10-year overall survival rates were similar in patients with or without dissemination (56). Therefore, extensive staging to assess dissemination is probably not necessary (57).

As *H. pylori* infection is prevalent in gastric MALT lymphoma cases, it is recommended that all patients should be treated with a course of eradication therapy, except those with t(11;18) or large-cell transformation (40). For localized disease, local treatment with either radiation therapy or surgery is effective (23,57). In patients with disseminated disease at presentation, chemotherapy should be used (57). MALT lymphomas have the best 5- and 10-year survival among all non-Hodgkin lymphoma subtypes in the study by the International Lymphoma Study Group (58). The 10-year survival rate of patients with low-grade MALT lymphoma was reported to be approximately 80% (20). Recent studies demonstrated a 5-year survival rate at 85% to 95% (59). Non-GI MALT lymphomas appear to progress more rapidly than GI MALT lymphomas (13).

REFERENCES

1. Isaacson PG, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue: a distinctive type of B-cell lymphoma. *Cancer*. 1983;52:1410–1416.
2. Isaacson PG, Wright DH. Extranodal malignant lymphoma arising from mucosa-associated lymphoid tissue. *Cancer*. 1984;53:2515–2524.
3. Spencer J, Finn T, Isaacson PG. Human Peyer's patches: an immunohistochemical study. *Gut*. 1986;27:405–410.
4. Dierlamm J, Pittaluga S, Wlodarska I, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. *Blood*. 1996;87:299–397.
5. Campo E, Miquet R, Krenascs L, et al. Primary nodal marginal zone lymphomas of splenic and MALT type. *Am J Surg Pathol*. 1999;23:59–68.
6. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
7. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of hematological malignancies. Report of the Clinical Advisory Committee meeting, Airlie House, Virginia, November 1997. *Mod Pathol*. 2000;13:193–207.
8. Isaacson PG, Chott A, Nakamura S, et al. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:214–217.
9. Isaacson PG. Mucosa-associated lymphoid tissue lymphoma. *Semin Hematol*. 1999;36:139–147.
10. Farinha P, Gascoyne RD. Molecular pathogenesis of mucosa-associated lymphoid tissue lymphoma. *J Clin Oncol*. 2005;23:6370–6378.
11. Zucca E, Conconi A, Pedrinis E, et al. Nongastric marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. *Blood*. 2003;101:2489–2495.
12. Isaacson PG, Müller-Hermelink HK, Piris MA, et al. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:157–160.
13. Thieblemont C, Bastion Y, Berger F, et al. Mucosa-associated lymphoid tissue gastrointestinal and nongastrointestinal lymphoma behavior: analysis of 108 patients. *J Clin Oncol*. 1997;15:1624–1630.
14. Kelley TW, Prayson RA, Barnett GH. Extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue arising in the lateral ventricle. *Leuk Lymphoma*. 2005;46:1423–1427.
15. Kumar S, Kumar D, Kaldjian EP, et al. Primary low-grade B-cell lymphoma of the dura: a mucosa-associated lymphoid tissue-type lymphoma. *Am J Surg Pathol*. 1997;21:81–87.
16. Kempton CL, Kurtin PJ, Inwards DJ, et al. Malignant lymphoma of the bladder: evidence from 36 cases that low-grade lymphoma of the MALT-type is the most common primary bladder lymphoma. *Am J Surg Pathol*. 1997;21:1324–1333.
17. Arnaoutakis K, Oo TH. Bronchus-associated lymphoid tissue lymphomas. *South Med J*. 2009;102:1229–1233.
18. Stefanovic A, Lossos IS. Extranodal marginal zone lymphoma of the ocular adnexa. *Blood*. 2009;114:501–510.
19. Burke JS. Extranodal lymphoid proliferations: general principles and differential diagnosis. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1165–1182.

20. de Jong D, Boot H, van Heerde P, et al. Histological grading in gastric lymphoma: pretreatment criteria and clinical relevance. *Gastroenterology*. 1997;112:1466–1474.
21. Burke JS. Are there site-specific differences among the MALT lymphomas—morphologic, clinical? *Am J Clin Pathol*. 1999;111(suppl 1):S133–S143.
22. Harris NL, Isaacson PG. What are the criteria for distinguishing MALT from non-MALT lymphoma at extranodal sites? *Am J Clin Pathol*. 1999;111(suppl 1):S126–S132.
23. Banks PM, Isaacson PG. MALT lymphomas in 1997. Where do we stand? *Am J Clin Pathol*. 1999;111(suppl 1):S75–S83.
24. Tomaszewski MM, Abbondanzo SL, Lupton GP. Extranodal marginal zone B-cell lymphoma of the skin: a morphologic and immunophenotypic study of 11 cases. *Am J Dermatopathol*. 2000;22:205–211.
25. Gupta D, Shidham V, Zemdhani V, et al. Primary bilateral mucosa-associated lymphoid tissue lymphoma of the breast with atypical ductal hyperplasia and localized amyloidosis. A case report and review of the literature. *Arch Pathol Lab Med*. 2000;124:1233–1236.
26. Isaacson PG, Dogan A, Price SK, et al. Immunoproliferative small-intestinal disease. An immunohistochemical study. *Am J Surg Pathol*. 1989;13:1023–1033.
27. Price SK. Immunoproliferative small intestinal disease: a study of 13 cases with alpha heavy-chain disease. *Histopathology*. 1990;17:7–17.
28. Mollejo M, Llorer E, Menarquez J, et al. Lymph node involvement by splenic marginal zone lymphoma. Morphological and immunohistochemical features. *Am J Surg Pathol*. 1997;21:772–780.
29. Zaer FS, Braylan RC, Zander DS, et al. Multiparametric flow cytometry in the diagnosis and characterization of low-grade pulmonary mucosa-associated lymphoid tissue lymphomas. *Mod Pathol*. 1998;11:525–532.
30. Isaacson PG, Watherspoon AC, Diss TC, et al. Bcl-2 expression in lymphomas. *Lancet*. 1991;337:175–176.
31. Watherspoon AC, Dogan A, Du MQ. Mucosa-associated lymphoid tissue lymphoma. *Curr Opin Hematol*. 2002;9:50–55.
32. Campo E, Jaffe ES. Nodal marginal zone B-cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:805–821.
33. Ferry JA, Yang WI, Zukerberg LR, et al. CD5+ extranodal marginal zone B-cell (MALT) lymphoma. A low grade neoplasm with a propensity for bone marrow involvement and relapse. *Am J Clin Pathol*. 1996;105:31–37.
34. Kost CB, Holden JT, Mann KP. Marginal zone B-cell lymphoma: a retrospective immunophenotypic analysis. *Clin Cytometry* 2008;74B:282–286.
35. Nathwani BN, Drachenberg MR, Hernandez AM, et al. Nodal monocytoid B-cell lymphoma (nodal marginal-zone B-cell lymphoma). *Semin Hematol*. 1999;36:128–138.
36. Aiello A, Giardini R, Tondini C, et al. PCR-based clonality analysis. A reliable method for the diagnosis and follow-up monitoring of conservatively treated gastric B-cell MALT lymphomas? *Histopathology*. 1999;34:326–330.
37. El-Zimaity HM, El-Zaatari FA, Dore MP, et al. The differential diagnosis of early gastric mucosa-associated lymphoma: polymerase chain reaction and paraffin section immunophenotyping. *Mod Pathol*. 1999;12:885–893.
38. Inagaki H. Mucosa-associated lymphoid tissue lymphoma: Molecular pathogenesis and clinicopathological significance. *Pathol Int*. 2007;57:474–484.
39. Vega F, Lin P, Medeiros J. Extranodal lymphomas of the head and neck. *Ann Diagn Pathol*. 2005;9:340–350.
40. Kahl BS. Update: gastric MALT lymphoma. *Curr Opin Oncol*. 2003;15:347–352.
41. Ye H, Dogan A, Karran L, et al. BCL 10 expression in normal and neoplastic lymphoid tissue. Nuclear localization in MALT lymphoma. *Am J Pathol*. 2000;157:1147–1154.
42. Zucca E, Bertoni F, Roggero E, et al. The gastric marginal zone B-cell lymphoma of MALT type. *Blood*. 2000;96:410–419.
43. Peng H, Diss T, Isaacson PG, et al. c-myc gene abnormalities in mucosa-associated lymphoid tissue (MALT) lymphomas. *J Pathol*. 1997;181:381–386.
44. Gaidano G, Volpe G, Pastore C, et al. Detection of BCL-6 rearrangements and p53 mutations in MALT-lymphoma. *Am J Hematol*. 1997;56:206–213.
45. Gaidano G, Capello D, Glohini A, et al. Frequent mutation of bcl-6 proto-oncogene in high-grade, but not low-grade, MALT lymphomas of the gastrointestinal tract. *Haematologica*. 1999;84:582–588.
46. Ye H, Nicholson AG, Dogan A, et al. BCL6 involved chromosomal translocation in MALT lymphoma of various sites. *Mod Pathol*. 2005;18:258A.
47. Joshi VV, Gagnon GA, Chadwick EG, et al. The spectrum of mucosa-associated lymphoid tissue lesions in pediatric patients infected with HIV. A clinicopathologic study of six cases. *Am J Clin Pathol*. 1997;107:592–600.
48. Swerdlow SH. Pediatric follicular lymphomas, marginal zone lymphomas, and marginal zone hyperplasia. *Am J Clin Pathol*. 2004;122(suppl):S98–S109.
49. Hussell T, Isaacson PG, Crabtree JE, et al. Helicobacter pylori-specific tumour-infiltrating T cells provide contact dependent help for the growth of malignant B cells in low-grade lymphoma of mucosa-associated lymphoid tissue. *J Pathol*. 1996;178:122–127.
50. Yamashita H, Watanabe H, Ajioka Y, et al. When can complete regression of low-grade gastric lymphoma of mucosa-associated lymphoid tissue be predicted after Helicobacter pylori eradication? *Histopathology*. 2000;37:131–140.
51. Chong EA, Svoboda J, Cherian S, et al. Regression of pulmonary MALT lymphoma after treatment with rituximab. *Leuk Lymphoma*. 2005;46:1383–1386.
52. Moslehi R, Devesa SS, Schairer C, et al. Rapidly increasing incidence of ocular non-Hodgkin lymphoma. *J Natl Cancer Inst*. 2006;98:936–939.
53. Asatiani E, Cohen P, Ozdemirli M, et al. Monoclonal gammopathy in extranodal marginal zone lymphoma (ENMZL) correlates with advanced disease and bone marrow involvement. *Am J Hematol*. 2004;77:144–146.
54. Wohrer S, Streubel B, Bartsch R, et al. Monoclonal immunoglobulin production is a frequent event in patients with mucosa-associated lymphoid tissue lymphoma. *Clin Cancer Res*. 2004;10:7179–7181.
55. Kalpadakis C, Pangalis GA, Vassilakopoulos TP, et al. Non-gastric extra-nodal marginal zone lymphomas—a single centre experience on 76 patients. *Leuk Lymphoma*. 2008;49:2308–2315.
56. Thieblemont C, Berger F, Dumontet C, et al. Mucosa-associated lymphoid tissue lymphoma is a disseminated disease in one third of 158 patients analyzed. *Blood*. 2000;95:802–806.
57. Thieblemont C. Clinical presentation and management of marginal zone lymphomas. *Hematology Am Soc Hematol Educ Program*. 2005;307–313.
58. Anon. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma: The Non-Hodgkin's Lymphoma classification Project. *Blood*. 1997;89:3909–3918.
59. Bacon CM, Du MQ, Degan A. Mucosa-associated lymphoid tissue (MALT) lymphoma: a practical guide for pathologists. *J Clin Pathol*. 2007;60:361–372.

CASE 28

Nodal Marginal Zone Lymphoma

CASE HISTORY

A 74-year-old man had a history of oligodendroma in the left frontal lobe for 14 years. Because of the patient's refusal, no surgical, radiation, or chemotherapy treatment was given. He only received dilantin and phenobarbital to control his petit mal seizures secondary to the brain tumor. In a routine check-up of dilantin level and complete blood count, he was found to have a total leukocyte count of 20,000/mL with 78% lymphocytes. A subsequent flow cytometric analysis of the peripheral blood identified a monoclonal B-cell population.

Physical examination revealed that the patient had bilateral axillary and left inguinal lymphadenopathy. A computed tomography scan also documented enlarged lymph nodes in the mediastinum and para-aortic site. However, he did not have hepatosplenomegaly. A biopsy was performed on the right axillary lymph node. Morphologic examination and flow cytometric analysis identified a small cell lymphoma. As dilantin is known to be able to induce lymphoma, this medication was discontinued. Nevertheless, because the patient had no B symptoms (fever, weight loss, and night sweats) and because the lymphoma was low-grade, no treatment of the lymphoma was given. The patient remained asymptomatic for 2 years without any sign of lymphoma progression. He finally died of unrelated illness.

FLOW CYTOMETRIC FINDINGS

Peripheral blood: CD5 36%, CD19 56%, CD19/CD5 0%, CD20 57%, CD23 13%, k 2%, l 56%, CD10 0%, CD14 0%.

Lymph node biopsy: CD5 0%, CD19 98%, CD19/CD5 0%, CD20 96%, CD23 4%, FMC-7 23%, k 0%, l 94%, CD10 4%, CD14 1% (Fig. 6.28.1).

DISCUSSION

Nodal marginal zone lymphoma (NMZL) was originally called monocytoid B-cell lymphoma (MBCL) (1), or para-follicular B-cell lymphoma (2). However, it was later found that MBCL was similar to lymphoma of the mucosa-associated lymphoid tissue (MALT) in morphology, immunophenotype, and cytogenetics (3,4). Since then, MBCL and MALT type lymphomas have been designated as nodal and extra-nodal marginal zone B-cell lymphomas, respectively, in the revised European-American Lymphoma and World Health Organization (WHO) classifications (5–7). Splenic marginal zone lymphoma is also similar to the above tumors, but their relationship is controversial (4). The frequency of NMZL is low, consisting of 1.8% of lymphoid neoplasms (6).

Morphology

The original description of MBCL includes three histologic patterns: the interfollicular and/or mantle zone–like pattern, the sinusoidal pattern, and the diffuse pattern (8). The last pattern is rare, and a diagnosis should be made with an immunologic confirmation (9). Campo et al. (3) described two histologic patterns in primary NMZL. The first one, designated splenic type, is characterized by a nodular proliferation of large and small lymphoid cells surrounding and infiltrating residual germinal centers (Figs. 6.28.2–6.28.4). The mantle corona is absent in most cases. The nodal architecture is totally effaced. The second pattern, designated MALT type, is characterized by a predominantly perivascular, perisinusoidal, and para-follicular infiltration. The mantle zone is preserved between the germinal centers and the tumor cells. Reactive follicles are always present. The MALT type was the predominant pattern in this study. However, both splenic and MALT types can be present in the same patient (10).

Nathwani et al. (9) emphasized that reactive follicles were present in >85% of their cases. However, their report did not elaborate the relationship between the presence of reactive follicles and the different tumor cell infiltration patterns (whether a sinus pattern, interfollicular pattern, or marginal zone pattern). Mollejo et al. (11), in contrast, emphasized the presence of marginal zone pattern in NMZL. Unlike the description by Campo et al. (3), their study indicated that the tumor cells were separated from the germinal center by a mantle zone in the “splenic type” (11). Traverse-Glehen et al. (12) emphasized that the splenic type of NMZL is distinguished from splenic marginal zone lymphoma in the absence of a biphasic pattern. In some cases, the germinal centers are invaded or totally replaced by the tumor cells; a process designated follicular colonization (4,9).

The cytology of this tumor is characterized by a polymorphic infiltration. The size of the tumor cells may be small, medium, or large. If both small cells and large blasts are present, the large cells are often present at the periphery of the nodules (3,13). Neutrophils and plasma cells are frequently present among the tumor cells. However, two major cell types are most frequently described (13,14). The monocytoid or marginal zone B cells are characterized by their abundant cytoplasm, centrally located bean-shaped or round nuclei, and fine chromatin pattern. The centrocyte-like cells have irregular nuclei and scant cytoplasm. When the marginal zone B cells show relatively scant cytoplasm, they can be recognized by the clear space separating each cell (9).

Other characteristic features of NMZL are frequent plasmacytic differentiation and the presence of composite lymphoma. In some cases, clusters and sheets of normal

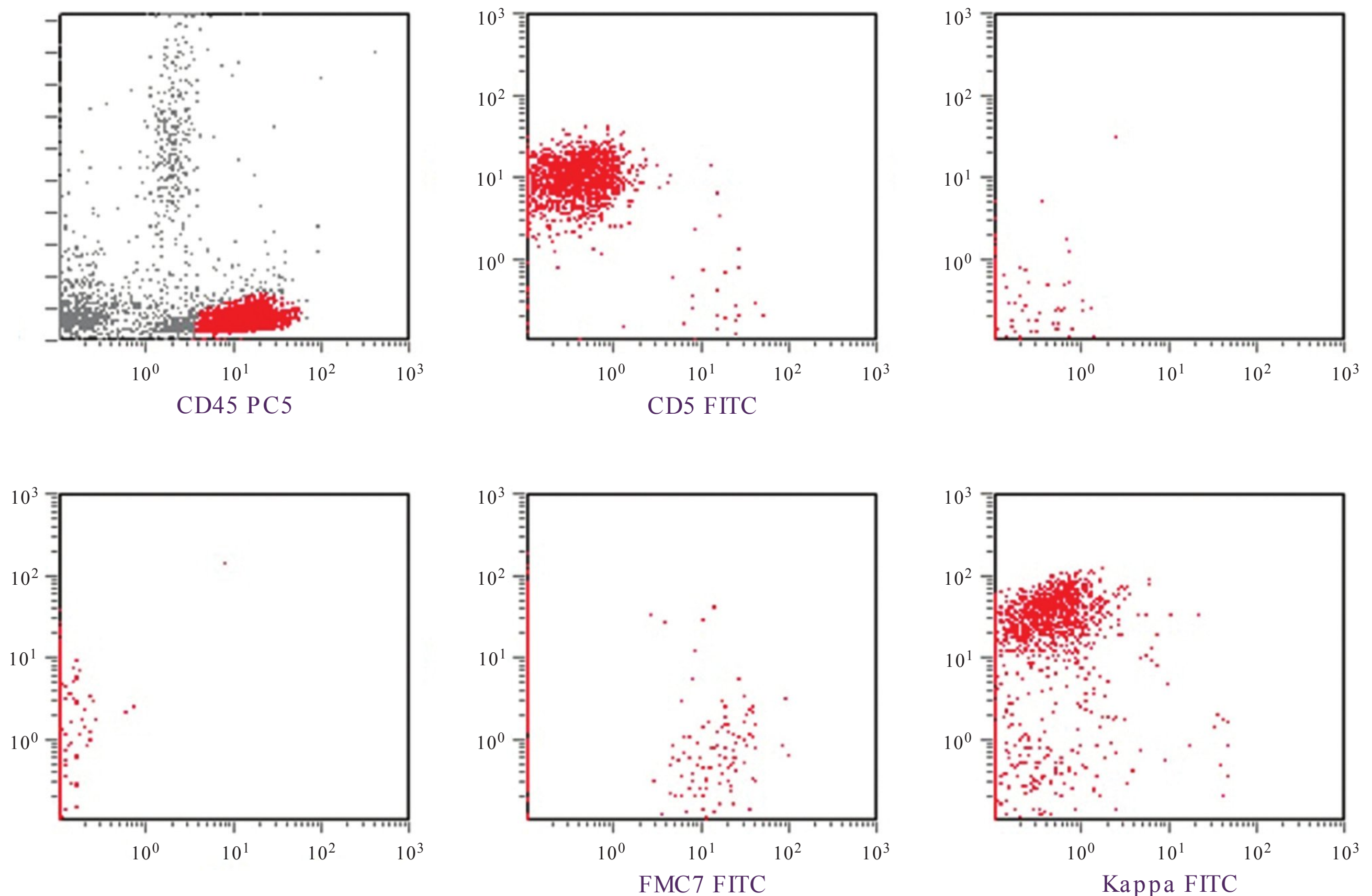


FIGURE 6.28.1 Flow cytometric analysis of the lymph node biopsy shows a CD19-positive monoclonal B-cell population. FMC-7 is partially positive, but CD10, CD23, and λ are negative. SS, side scatter; PC5, phycoerythrin-cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; RD1, rhodamine.

and abnormal plasma cells become the dominant feature that resembles a plasmacytoma or lymphoplasmacytic lymphoma. Monoclonal gammopathy may be present in those cases. However, the presence of monocytoid B-cell features or a nodular pattern in NMZL may help to exclude the diagnosis of lymphoplasmacytic lymphoma (9,12). The composite lymphoma is frequently composed of NMZL and follicular lymphoma. Composite NMZL with Hodgkin lymphoma (15) and with plasma cell myeloma (16) have also been reported recently. Cases with a high percentage of large tumor cells are sometimes considered a composite lymphoma. However, some authors consider that this so-called composite lymphoma may simply represent the heterogeneity of the tumor, and prefer to call it a large cell variant (17).

A floral variant of NMZL has been reported recently (18). It is similar to the floral variant of follicular lymphoma in the presence of a prominent mantle zone forming a scalloped outline. In some cases, a clear zone is present around the mantle zone. Five of the six cases reported involved cervical lymph nodes. All patients were asymptomatic with a stage I disease and showed no recurrence after surgical excision of the lymph nodes (18). In children and young adults, disruption of residual follicles resembling progressive transformation of germinal centers is a common feature in NMZL and is similar to the floral variant of NMZL (19). The 2008 WHO classification has separated pediatric NMZL from the

adult form, not only because of its distinct histology but also because of its indolent clinical course and favorable prognosis (7,18,20). However, features of pediatric NMZL have also been found in adult patients, the fact that challenges the appropriateness of this designation (21).

NMZL has a propensity to transform into large cell lymphomas (7,22). High-grade transformation may be seen in 20% of NMZL cases and can be recognized by the presence of one or more well-defined monomorphic clusters or sheets of large B cells or when >20% of large cells are mixed with the smaller tumor cells (9). The transformed large tumor cells also show a high mitotic index and high Ki-67 labeling (13). The characteristic morphologic features of NMZL are summarized in Table 6.28.1.

Differential diagnosis includes mantle cell lymphoma, small lymphocytic lymphoma, and follicular lymphoma; all of which may occasionally show a marginal zone distribution (9,13). Mantle cell lymphoma usually shows monotonous cellular proliferation, absence of large blasts, and absence of plasma cell differentiation (3,11,23). Distinction from follicular lymphoma is mainly based on the benign nature of the reactive follicles and residual germinal centers in NMZL as determined by morphology and immunophenotyping—namely, negative bcl-2 (Figs. 6.28.5 and 6.28.6) but a high percentage of Ki-67 (11). The lack of clusters of prolymphocytes and

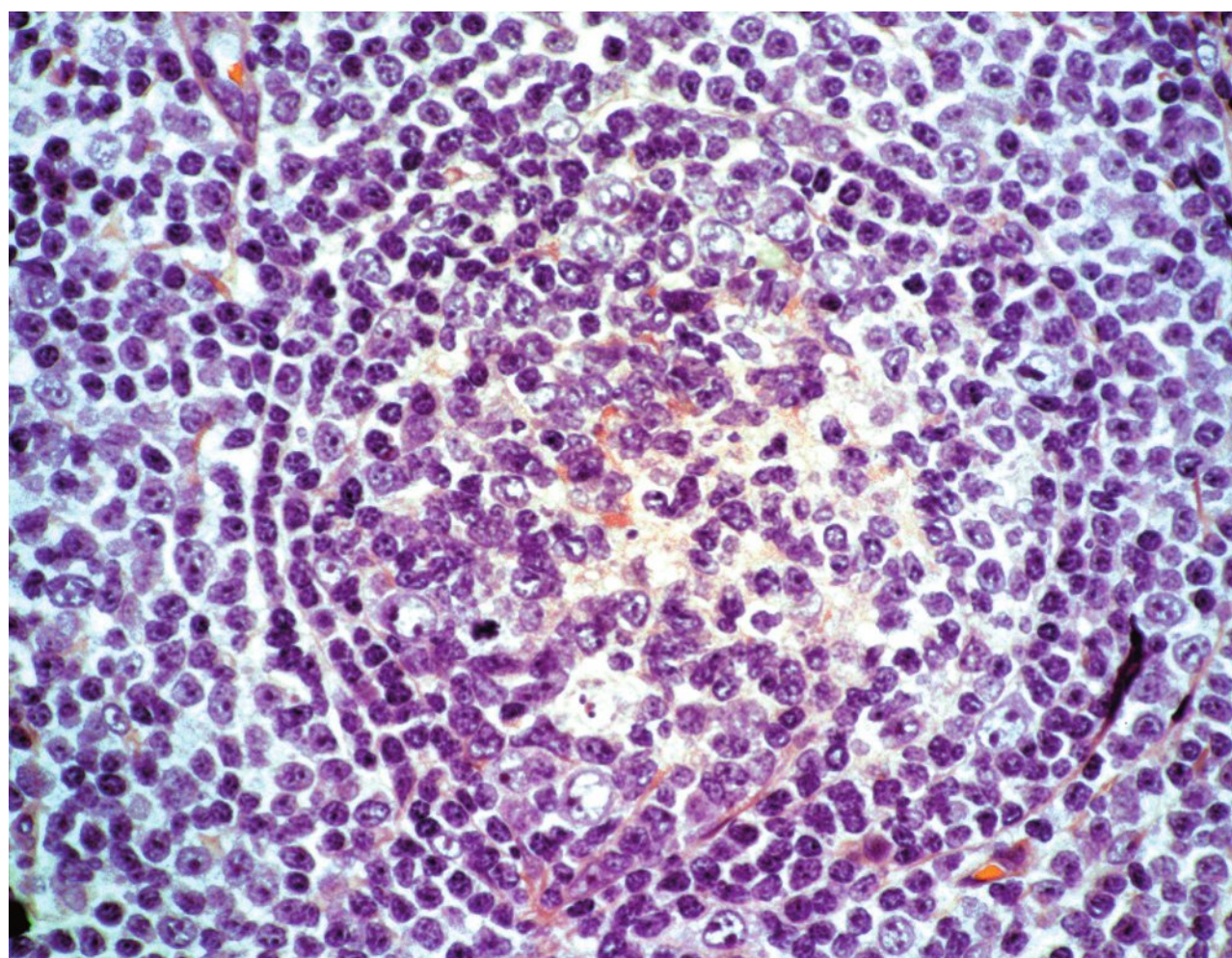


FIGURE 6.28.2 Lymph node biopsy shows monocytoid B-cell infiltration surrounding a germinal center with a well-preserved mantle zone. The tumor cells have immature chromatin pattern and abundant cytoplasm. There are some large cells intermingled with the small cells. Hematoxylin and eosin, 40× magnification.

paraimmunoblasts and the immunophenotype may help distinguish small lymphocytic lymphoma from NMZL (3).

Monocytoid B-cell hyperplasia also should be distinguished from NMZL (Fig. 6.28.7). The malignant nature of NMZL can be recognized by the prominent confluent proliferation in the tissue, nuclear irregularity, high mitotic figures, and more transformed large cells (24). The tumor cells are bcl-2 and CD43 positive, but bcl-2 and CD43 are negative in benign monocytoid B cells (13,25). The final resort is to demonstrate monoclonality by flow cytometry and/or polymerase chain reaction (25).

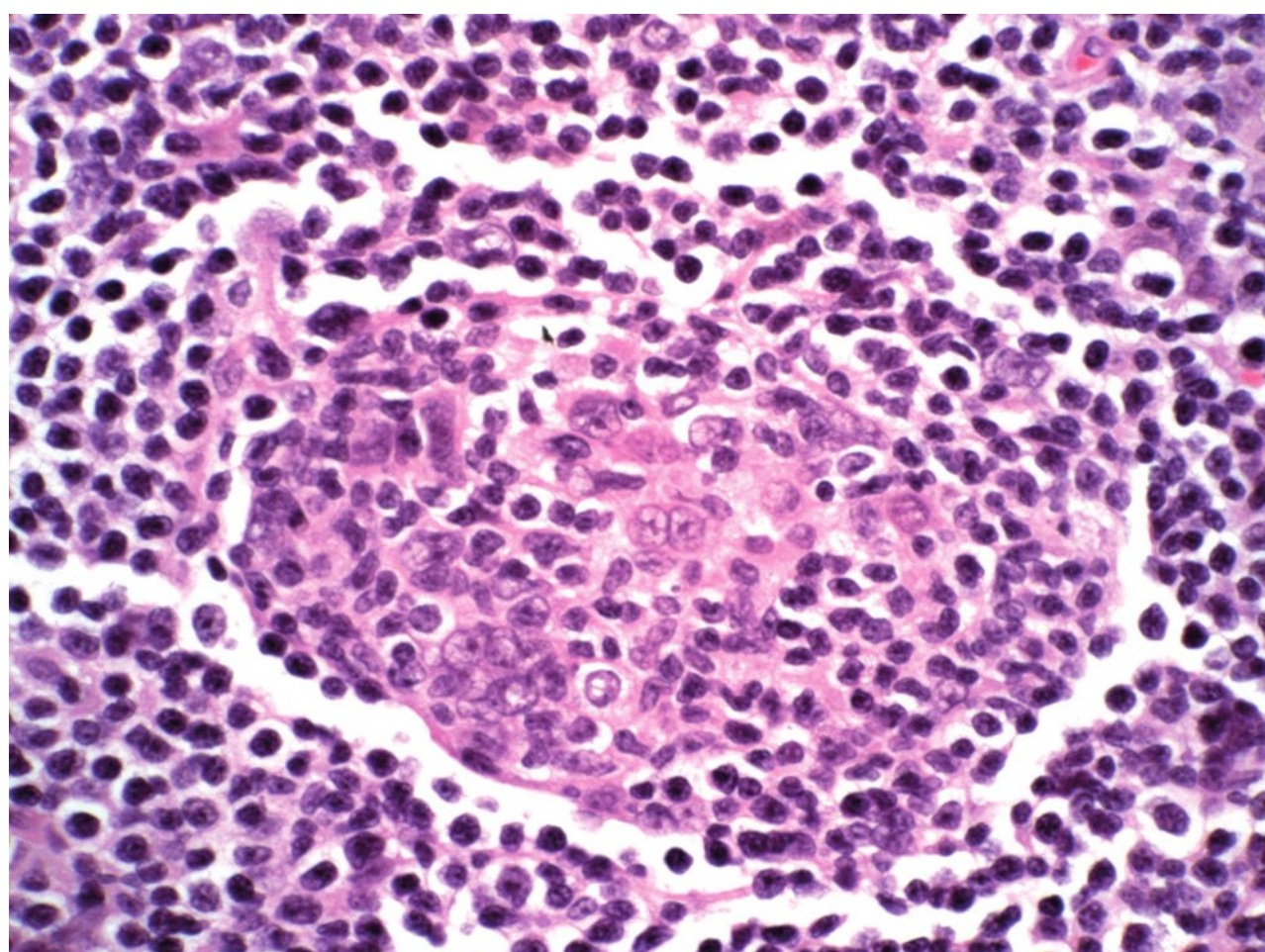


FIGURE 6.28.3 Lymph node biopsy reveals a germinal center surrounded by centrocyte-like tumor cells, which are small with irregular nuclei and dense chromatin pattern. A mantle zone is absent. The residual germinal center is partially colonized. Hematoxylin and eosin, 40× magnification.

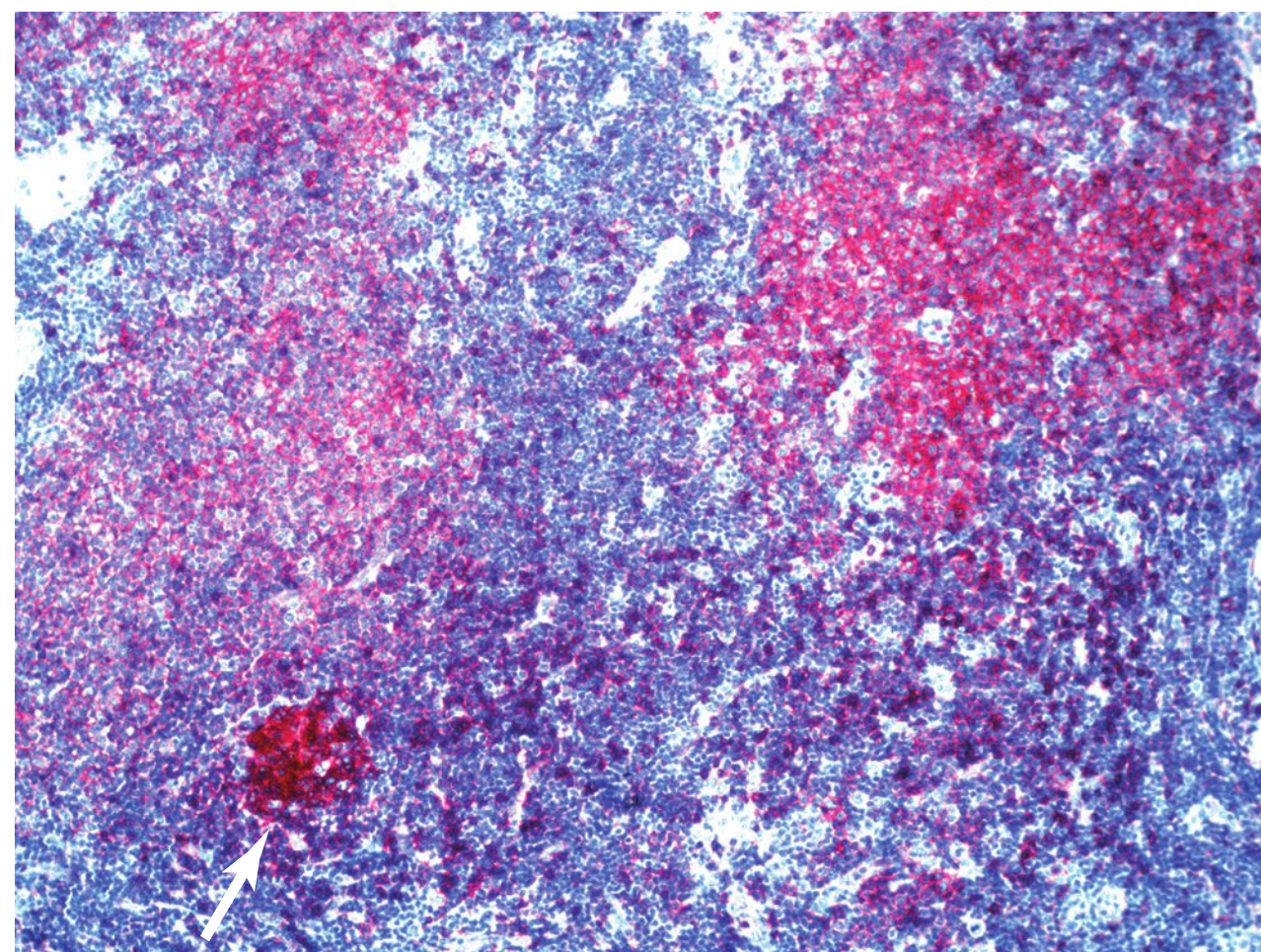


FIGURE 6.28.4 Lymph node biopsy shows that CD20 (B-cell marker) staining highlights the nodular aggregates of tumor cells. A residual germinal center reveals darker staining (white arrow). Immunoperoxidase, 10× magnification.

Immunophenotype

The immunophenotype of NMZL is that of a monoclonal B-cell proliferation—namely, the presence of CD19, CD20, CD22, CD79a, and a monoclonal k or l pattern (6,10,17,26). NMZL is also positive for BCL-2. CD43 coexpression is only seen in 50% of cases in general (7), but in 80% of pediatric cases (20). CD45RA, CD74 (LN2), and CD75 (LN1) have also been detected in some cases (13). However, the characteristic findings are rather the absence of CD5, CD10, CD23, cyclin D1 and BCL-6, which may help to distinguish NMZL from other non-Hodgkin lymphomas (7). For instance, CD5 and CD23 are positive in small lymphocytic lymphoma, while CD5 and cyclin D1 are positive in mantle cell lymphoma (9). CD10 and BCL-6 are positive in follicular lymphoma (9). CD23 may also help highlight the residual germinal centers, in case of follicular colonization in NMZL (9).

A follicular dendritic cell meshwork can be demonstrated by CD21 in NMZL (9,11). Immunoglobulin D (IgD) is

TABLE 6.28.1

Characteristic Morphologic Features of NMZL

Histologic pattern	Nodular proliferation around germinal center (splenic type) and perivascular, perisinusoidal, and parafollicular infiltration (MALT type)
Cytology	Polymorphic population with major components of monocytoid B cells or centrocyte-like cells
Specific features	Combination of histologic pattern and cytology

NMZL, nodal marginal zone lymphoma; MALT, mucosa-associated lymphoid tissue.

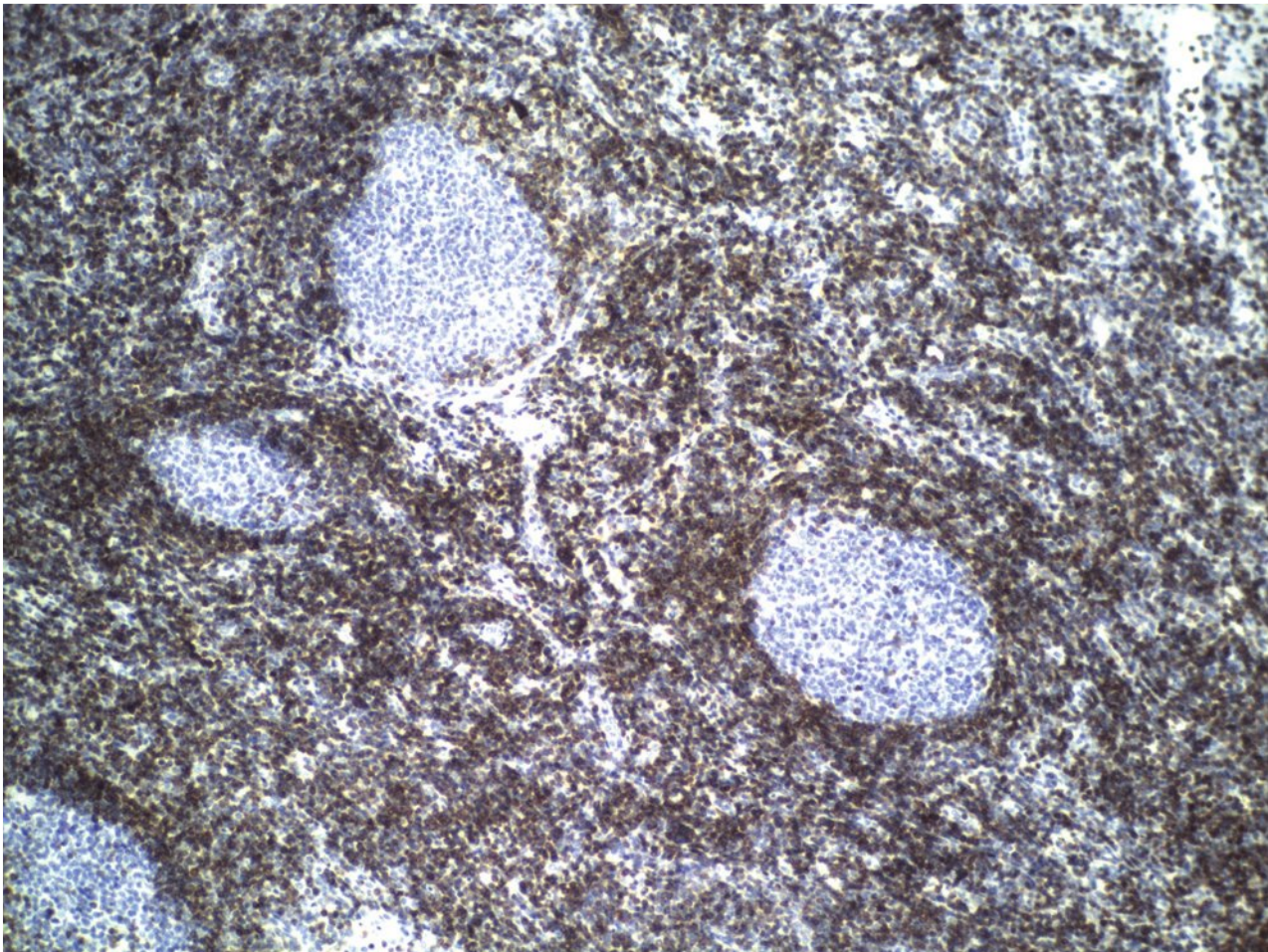


FIGURE 6.28.5 Lymph node biopsy shows BCL-2 staining of the cells in the expanded marginal zone, but the residual germinal center is devoid of staining, excluding follicular lymphoma. Immunoperoxidase, 10× magnification.

positive in some reports (11) but negative in others (26). Campo et al. (3) and Campo and Jaffe (13) found that IgD is positive in the splenic subtype but negative in the MALT subtype of NMZL. Because IgD is positive for mantle zone cells but negative for marginal zone cells, the positive reaction in the splenic subtype of NMZL and the splenic marginal zone lymphoma argues against a marginal zone derivative for these tumors (3).

Bcl-2 is positive for tumor cells in most cases, and its absence in the residual germinal center is helpful in excluding follicular lymphoma (11,13). In contrast to follicular lymphoma, bcl-2 gene is not rearranged in NMZL (4,17,26).

In the current case, the lymphoma was suspected to be induced by the long-term dilantin treatment. Although the patient had multiple lymphadenopathy and peripheral

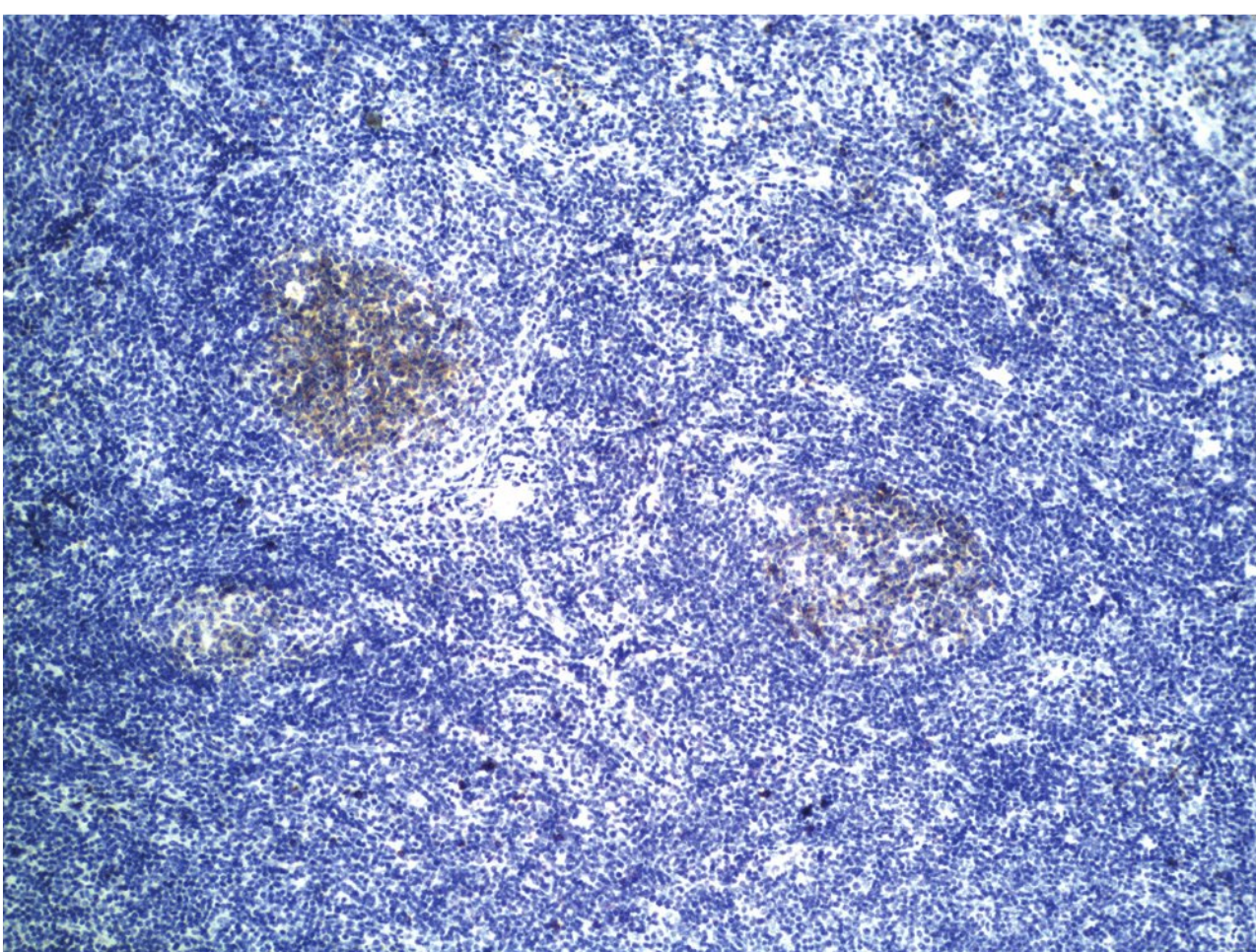


FIGURE 6.28.6 Lymph node biopsy shows that CD10 stain identifies the residual germinal center, which is negative for BCL-2 in Figure 6.28.5. Immunoperoxidase, 10× magnification.

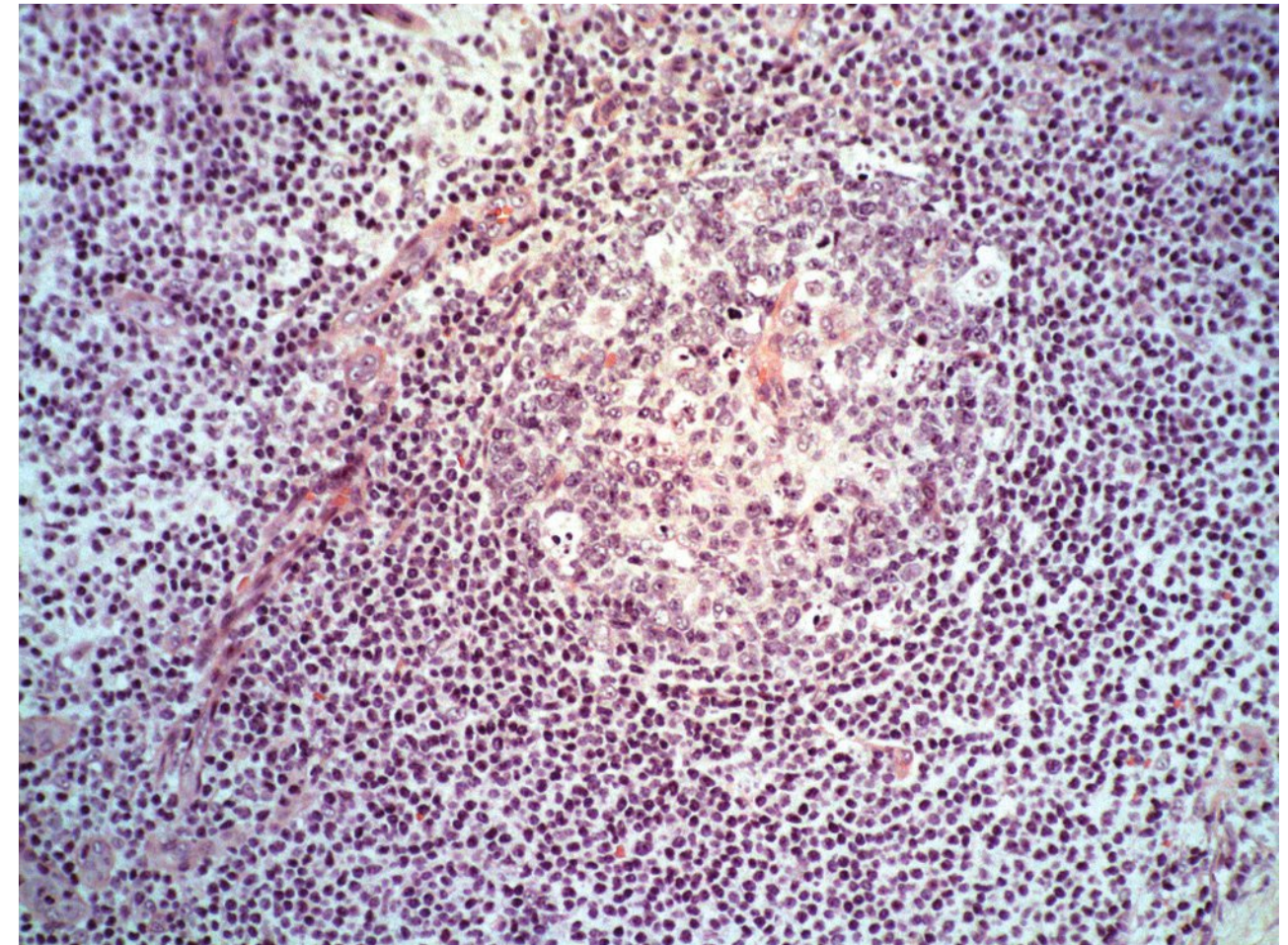


FIGURE 6.28.7 Lymph node biopsy reveals monocytoid B-cell proliferation surrounding a germinal center, mimicking the pattern of nodal marginal zone B-cell lymphoma. These monocytoid B-cells are bland looking, with no mitosis and large cells. Most part of the lymph node shows normal architecture. Hematoxylin and eosin, 20× magnification.

blood involvement, he did not show signs of disease progression in 2 years. Unfortunately, he died of unrelated illness at the end of 2 years, so no long-term follow-up was achieved. For some unexplained reason, patients with NMZL frequently have para-aortic lymph node involvement (9), as with this patient. The immunophenotype of the peripheral blood and lymph node was consistent with NMZL. Notably, CD10 and CD23 are negative and no dual CD19/CD5 stain was identified.

Comparison of Flow Cytometry and Immunohistochemistry

The immunophenotypes as demonstrated by both flow cytometry and immunohistochemistry are not specific for the diagnosis of NMZL. However, it may help to establish the diagnosis by excluding other non-Hodgkin lymphomas. In this regard, immunohistochemical staining is more helpful than flow cytometry as it can distinguish tumor cells from residual follicles. For instance, CD21, CD23, and CD35 are positive in colonized follicles (6,10), but the positive staining for these surface antigens may be mistaken as the markers for the tumor cells. The absence of BCL-2 in the follicles distinguishes residual germinal centers from follicular lymphoma. Similar to hairy cell leukemia, DBA-44 has been demonstrated in some cases of NMZL (27).

Molecular Genetics

Immunoglobulin (Ig) gene rearrangements are present in NMZL. Most of the common oncogenes, such as BCL-1, BCL-2, BCL-3, and BCL-6 are negative in NMZL (4,14,17,26). C-MYC rearrangement is also not present in NMZL (4).

Analysis of Ig variable heavy chain (V_H) usage and mutation patterns has proved that NMZL is a heterogeneous tumor, arising from different subsets of marginal zone B lymphocytes (27–29). In other words, the tumor cells seem to arise from virgin B cells that express unmutated V_H

genes, memory B cells that show somatic mutations, and germinal center B cells that undergo somatic hypermutation (29). The pattern of somatic mutation and the V_H gene segment usage were found to be different between NMZL and splenic marginal zone B-cell lymphoma, suggesting that these two diseases are distinct entities (12). Another study showed that the use of V_H gene differed between hepatitis C virus–positive and –negative NMZL cases, and in both groups there was evidence of a clonal antigen-positive selection driven by antigens (30). In other words, the tumor cell proliferation is probably due to hepatitis C virus and, in the other group, an unknown pathogen stimulation.

Trisomy 3 is the most frequent cytogenetic aberration in NMZL, occurring in 56% to 78% of cytogenetically abnormal cases (14,17). Trisomy 18 was the second most common, reported in 21% of MALT lymphoma cases (31) and in 25% of non-MALT type marginal zone lymphoma cases (17). In a study of 23 cases of pediatric NMZL, 4 were positive for trisomy 18 and 1 case showed trisomy 3 (20). Structural abnormalities of chromosome 1 is a relatively common finding (4,9). Translocation t(11;18)(q21;q21) has also been reported in some cases of NMZL and is the most common translocation in extranodal low-grade MALT lymphoma (13). This translocation leads to a fusion of apoptosis inhibitor-2 (API2) gene on chromosome 11 and MALT lymphoma-associated translocation (MLT) gene in chromosome 18 (32). However, a study with reverse transcription-polymerase chain reaction (RT-PCR) and genomic long and accurate PCR analysis revealed that none of the 9 NMZL cases had API2 and/or MLT, whereas 17 of 95 MALT lymphoma cases were positive (33). It appears that NMZL and MALT share the same chromosomal abnormalities, but the frequencies of these anomalies differ in each entity (4,14,34).

There is a variety of numeric cytogenetic abnormalities identified in marginal zone B-cell lymphoma by karyotyping and fluorescence in situ hybridization, but they are of rare occurrence (11,30). Many of these abnormalities are included in complex karyotypes (34). Some cytogenetic abnormalities, such as trisomy 12 and t(14;18), are probably associated with a composite lymphoma containing small lymphocytic lymphoma or follicular lymphoma, but they are not specific for NMZL (13). A genome-wide array-based comparative genomic hybridization study of 7 NMZL cases showed neither recurrent genome alterations nor any change in NALT1 gene copy number (35). The salient features for laboratory diagnosis of NMZL are summarized in Table 6.28.2.

Clinical Manifestations

Patients with NMZL are usually diagnosed at age 60 to 65 years, with a male-to-female ratio of 1:5 (13). The lymph nodes involved are usually in the head and neck, and less frequently in the inguinal and retroperitoneal areas. Patients are usually asymptomatic, but fever, night sweats, and weight loss can be occasionally seen in patients with tumor progression. NMZL is frequently associated with autoimmune diseases, including Sjögren syndrome, systemic lupus erythematosus, and Raynaud phenomenon (22,36). Hepatitis C virus infection is frequently associated with NMZL with an incidence varying from 20% to 40% (7,37). This frequency is the highest among other non-Hodgkin lymphomas (37).

TABLE 6.28.2

Salient Features for Laboratory Diagnosis of NMZL

1. Positive for CD19, CD20, CD21, CD22, CD43, CD79a and BCL-2 in a monoclonal population
2. Negative for CD5, CD10, CD23, cyclin D1 and BCL-6
3. IgD can be positive or negative, depending on the tissue type.
4. Rearrangement of Ig genes
5. No rearrangement for BCL-1, BCL-2, BCL-3, and BCL-6
6. Trisomy 3 and trisomy 18 are the most common cytogenetic abnormalities.

NMZL, nodal marginal zone lymphoma; Ig, immunoglobulin.

The pediatric NMZL differs from the adult form in its male predominance and indolent clinical course (asymptomatic and localized) (7,19,20). It usually involves the head and neck lymph nodes. The prognosis is excellent, the relapse rate is low and patients survive long after conservative treatment (7,19,20).

Bone marrow involvement has been observed in 32% of patients in one study series (38). Primary NMZL, by definition, is not accompanied by splenomegaly. It may spread through lymphatics to other lymph nodes, but peripheral blood is seldom involved (17). However, a leukemic subtype of marginal zone lymphoma with only blood and bone marrow involvement has recently been identified (39). A comparative study of three types of marginal zone B-cell lymphomas showed that MALT lymphoma usually had stage I disease, NMZL showed stage II or III disease at diagnosis, and splenic marginal zone lymphoma was presented with stage IV disease (4). However, NMZL in children and young adults usually presents with stage I disease (19).

Although NMZL is a localized disease, its progression is rapid, with a median progression time of 1 year, as compared with >5 years in the splenic marginal zone lymphoma in one study (17). However, the survival time for both types of lymphoma is comparable (median survival 9 years). Recently, Camacho et al. (27) showed that a shorter, failure-free survival is associated with loss of survivin and active caspase 3 in the tumor cells of NMZL.

REFERENCES

1. Sheibani K, Sohn C, Burke JS, et al. Monocytoid B-cell lymphoma: a novel B-cell neoplasm. *Am J Pathol.* 1986;124:310–318.
2. Cousar JB, McGinn DL, Click AD, et al. Report of an unusual lymphoma arising from parafollicular B-lymphocytes (PBLs) or so-called ‘monocytoid’ lymphocytes. *Am J Clin Pathol.* 1987;87:121–128.
3. Campo E, Miquet R, Krenacs L, et al. Primary nodal marginal zone lymphomas of splenic and MALT type. *Am J Surg Pathol.* 1999;23:59–68.

4. Dierlamm J, Pittaluga S, Wlodarska I, et al. Marginal zone B-cell lymphomas of different sites share similar cytogenetic and morphologic features. *Blood*. 1996;87:299–307.
5. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
6. Isaacson PG, Nathwani BN, Piris MA, et al. Nodal marginal zone B-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:161.
7. Campo E, Pileri SA, Jaffe ES, et al. Nodal marginal zone lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:218–219.
8. Sheibani K. Monocytoid B-cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. Baltimore, MD: Williams & Wilkins; 1992:629–644.
9. Nathwani BN, Drachenberg MR, Hernandez AM, et al. Nodal monocytoid B-cell lymphoma (nodal marginal zone B-cell lymphoma). *Semin Hematol* 1999;36:128–138.
10. Maes B, De Wolf-Peeters C. Marginal zone cell lymphoma—an update on recent advances. *Histopathology*. 2002;40:117–126.
11. Mollejo M, Lloret E, Menarguez J, et al. Lymph node involvement by splenic marginal zone lymphoma: morphological and immunohistochemical features. *Am J Surg Pathol*. 1997;21:772–780.
12. Traverse-Glehen A, Davi F, Simon EB, et al. Analysis of V_H genes in marginal zone lymphoma reveals marked heterogeneity between splenic and nodal tumors and suggests the existence of clonal selection. *Haematologica*. 2005;90:470–478.
13. Campo E, Jaffe ES. Nodal marginal zone B-cell lymphomas. In: Knowles DM, ed. *Neoplastic Hematopathology*. Philadelphia, PA: Lippincott, Williams & Wilkins; 2001:805–821.
14. De Wolf-Peeters C, Pittaluga S, Dierlamm J, et al. Marginal zone B-cell lymphomas including mucosa-associated lymphoid tissue type lymphoma (MALT), monocytoid B-cell lymphoma and splenic marginal zone cell lymphoma and their relation to the reactive marginal zone. *Leuk Lymphoma*. 1997;26:467–478.
15. Zettl A, Rüdiger T, Marx A, et al. Composite marginal zone B-cell lymphoma and classical Hodgkin's lymphoma: a clinicopathological study of 12 cases. *Histopathology*. 2005;46:217–228.
16. Saito H, Oka K, Nakamura N, et al. A common clonal origin of nodal marginal zone B-cell lymphoma and plasma cell myeloma demonstrating different immunophenotypes: a case report of composite lymphoma. *Diagn Mol Pathol*. 2004;13:75–80.
17. Berger F, Felman P, Thieblemont C, et al. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. *Blood*. 2000;95:1950–1956.
18. Karube K, Ohshima K, Tsuchiya T, et al. A “floral” variant of nodal marginal zone lymphoma. *Hum Pathol*. 2005;36:202–206.
19. Taddesse-Heath L, Pittaluga S, Sorbara L, et al. Marginal zone B-cell lymphoma in children and young adults. *Am J Surg Pathol*. 2003;27:522–531.
20. Rizzo KA, Streubel B, Pittaluga S, et al. Marginal zone lymphomas in children and the young adult population; characterization of genetic aberrations by FISH and RT-PCR. *Mod Pathol*. 2010;23:866–873.
21. Gitelson E, Al-Saleem T, Robu V, et al. Pediatric nodal marginal zone lymphoma may develop in the adult population. *Leuk Lymphoma*. 2010;51:89–94.
22. Shin SS, Sheibani K. Monocytoid B-cell lymphoma. *Am J Clin Pathol*. 1993;99:421–425.
23. Pittaluga S, Verhoef G, Criel A, et al. “Small” B-cell non-Hodgkin's lymphomas with splenomegaly at presentation are either mantle cell lymphoma or marginal zone cell lymphoma: a study based on histology, cytology, immunohistochemistry and cytogenetic analysis. *Am J Surg Pathol*. 1996;20:211–223.
24. Nathwani BN, Mohrmann RL, Brynes RK, et al. Monocytoid B-cell lymphomas: an assessment of diagnostic criteria and a perspective on histogenesis. *Hum Pathol*. 1992;23:1061–1071.
25. Kojima M, Motoori T, Iijima M, et al. Florid monocytoid B-cell hyperplasia resembling nodal marginal zone B-cell lymphoma of mucosa associated lymphoid tissue type. A histological and immunohistochemical study of four cases. *Pathol Res Pract*. 2006;202:877–882.
26. Fisher R, Dahlberg S, Nathwani B, et al. A clinical analysis of two indolent lymphoma entities: mantle cell lymphoma and marginal zone lymphoma (including the mucosa-associated lymphoid tissue and monocytoid B-cell subcategories). A Southwest Oncology Group Study. *Blood*. 1995;85:1075–1082.
27. Camacho FI, Algara P, Mollejo M, et al. Nodal marginal zone lymphoma: a heterogeneous tumor: a comprehensive analysis of a series of 27 cases. *Am J Surg Pathol*. 2003;27:762–771.
28. Tierens A, Delabie J, Pittaluga S, et al. Mutation analysis of the rearranged immunoglobulin heavy chain genes of marginal zone cell lymphomas indicated an origin from different marginal zone B lymphocytes subsets. *Blood*. 1998;91:2381–2386.
29. Conconi A, Bertoni F, Pedrinis E, et al. Nodal marginal zone B-cell lymphomas may arise from different subsets of marginal zone B lymphocytes. *Blood*. 2001;98:781–786.
30. Marasca R, Vaccari P, Luppi M, et al. Immunoglobulin gene mutations and frequent use of VH1-69 and VH4-34 segments in hepatitis C virus-positive and hepatitis C virus-negative nodal marginal zone B-cell lymphoma. *Am J Pathol*. 2001;159:253–261.
31. Wotherspoon AC, Finn TM, Isaacson PG. Trisomy 3 in low-grade B-cell lymphomas of mucosa-associated lymphoid tissue. *Blood*. 1995;85:2000–2004.
32. Dierlamm J, Baens M, Stefanova-Ouzounova M, et al. Detection of t(11;18)(q21;q21) by interphase fluorescence in situ hybridization using API2 and MLT specific probes. *Blood*. 2000;96:2215–2218.
33. Yonezumi M, Suzuki R, Suzuki H, et al. Detection of API2-MALT1 chimeric gene in extranodal and nodal marginal zone B-cell lymphoma by reverse transcription polymerase chain reaction (PCR) and genomic long and accurate PCR analyses. *Br J Haematol*. 2001;115:588–594.
34. Cuneo A, Bigoni R, Roberti MG, et al. Molecular cytogenetic characterization of marginal zone B-cell lymphoma: correlation with clinicopathologic findings in 14 cases. *Hematologica*. 2001;86:64–70.
35. Kim WS, Honma K, Karnan S, et al. Genome-wide array-based comparative genomic hybridization of ocular marginal zone B cell lymphoma: comparison with pulmonary and nodal marginal zone B cell lymphoma. *Genes Chromosomes Cancer*. 2007;46:776–783.
36. Ngan BY, Warnke RA, Wilson M, et al. Monocytoid B-cell lymphoma: a study of 36 cases. *Hum Pathol*. 1991;22:409–421.
37. Acaini L, Lucioni M, Boveri E, et al. Nodal marginal zone lymphoma: current knowledge and future directions of an heterogeneous disease. *Eur J Haematol*. 2009;83:166–174.
38. Nathwani BN, Anderson JR, Armitage JO, et al. Marginal zone B-cell lymphoma: a clinical comparison of nodal and mucosa-associated lymphoid tissue types. *J Clin Oncol*. 1999;17:2486–2492.
39. Tam CS, Prince HM, Westerman D, et al. Leukaemic subtype of marginal zone lymphoma: a presentation of three cases and literature review. *Leuk Lymphoma*. 2004;45:705–710.

CASE 29

Follicular Lymphoma

CASE HISTORY

A 50-year-old man was admitted for further evaluation of lymphoma after chemotherapy. The patient noticed lumps on both sides of his groin 1 year ago and was considered to have an inguinal hernia. A hernia belt helped relieve the pain, but the lumps continued to grow. His private physician then suggested a biopsy, which showed lymphoma. He underwent four cycles of chemotherapy with minimal response. Instead, he noticed an increase in size of bilateral inguinal lymph nodes.

Physical examination on admission revealed lymphadenopathy in the supraclavicular, axillary, and inguinal regions, but the cervical lymph node was not enlarged. The liver and spleen were not palpable. His blood chemistry was unremarkable except for an elevated lactate dehydrogenase (LDH) (411 U/L). Liver and renal function tests were within normal limits.

Computed tomography scan revealed multiple enlarged lymph nodes in the mediastinal and retroperitoneal regions. The spleen was also enlarged to 14×6 cm.

The biopsy of the left inguinal lymph node confirmed the previous diagnosis of follicular lymphoma (FL) with large-cell infiltration in the interfollicular area. A bone marrow biopsy showed multiple lymphoid aggregates in the paratrabecular areas.

FLOW CYTOMETRY FINDINGS

Lymph node biopsy: CD5 18%, CD19 71%, CD19/CD10 30%, CD20 96%, CD20/bcl-2 69%, CD23 9%, FMC-7 86%, CD19/k 74%, CD19/l 2%.

Bone marrow biopsy: CD5 7%, CD19 63%, CD19/CD10 45%, CD20 92%, CD20/bcl-2 76%, CD23 11%, FMC-7 78%, CD19/k 73%, CD19/l 0% (Fig. 6.29.1).

IMMUNOHISTOCHEMICAL FINDINGS

The malignant follicles in the lymph node showed positive staining for CD20, bcl-2, and bcl-6 but negative staining for CD3 and CD5.

CYTOGENETIC FINDINGS

Cytogenetic analysis showed a balanced translocation between chromosomes 14 and 18 in all cells analyzed. Trisomy 21 was also noted in the abnormal clone.

DISCUSSION

FL is one of the most common types of non-Hodgkin lymphoma, accounting for 20% to 30% of all non-Hodgkin lymphoma and 40% to 50% of adult non-Hodgkin lymphoma (1). In European countries, the incidence ranges from 13% to 22% (1,2). However, the incidence of FL in Asia, including Japan, is relatively low (3.8% to 12%) (1,3,4).

The term FL was originally used in the Working Formulation (5). In the Kiel classification, this lymphoma is called centroblastic-centrocytic lymphoma (6). The Revised European–American Lymphoma classification changed the name to follicle center lymphoma (7), but the World Health Organization (WHO) scheme retains the original term, FL (8–10).

Morphology

In FL, the normal architecture of the lymph node is usually partially or completely effaced (Table 6.29.1). The neoplastic follicles are often uniform in both size and shape (1,9–12). These follicles are evenly distributed, frequently with a back-to-back pattern so that the density of follicles is higher than that in follicular hyperplasia (FH), and there is sparse intervening interfollicular tissue. The margin of the tumor follicles is usually poorly defined. The mantle zone is usually absent or attenuated. In some cases, the follicular pattern is very subtle and requires immunophenotyping to substantiate the diagnosis. The capsule of the lymph node is frequently infiltrated by the lymphoma cells, which may spread into the perinodal soft tissue. The subcapsular and medullary sinuses are often obliterated by the tumor infiltrate.

Cytologically, FL is composed of small cleaved lymphocytes (centrocytes) and large transformed lymphoid cells (centroblasts) in varying proportions, but it usually shows a monomorphic appearance without a polarization pattern in the follicle. In most cases of FL, the mitotic rate is low and tangible-body macrophages are seldom seen except for the large-cell subtype. The presence of atypical lymphoid cells in the interfollicular region is helpful for the diagnosis of FL, although inflammatory cells may also be present.

In FH, the nodal architecture is usually well preserved (Table 6.29.2) (13). The hyperplastic follicles are more irregular in size, shape, and distribution than are the follicles of FL. In addition, the benign follicles have lower density than the malignant ones. The demarcation between the germinal center and mantle zone is distinct, and the mantle zone is intact. The capsule of the lymph node is usually not involved in FH.

Cytologic features may further distinguish FH from FL. In FH, the germinal centers contain mixed large and small

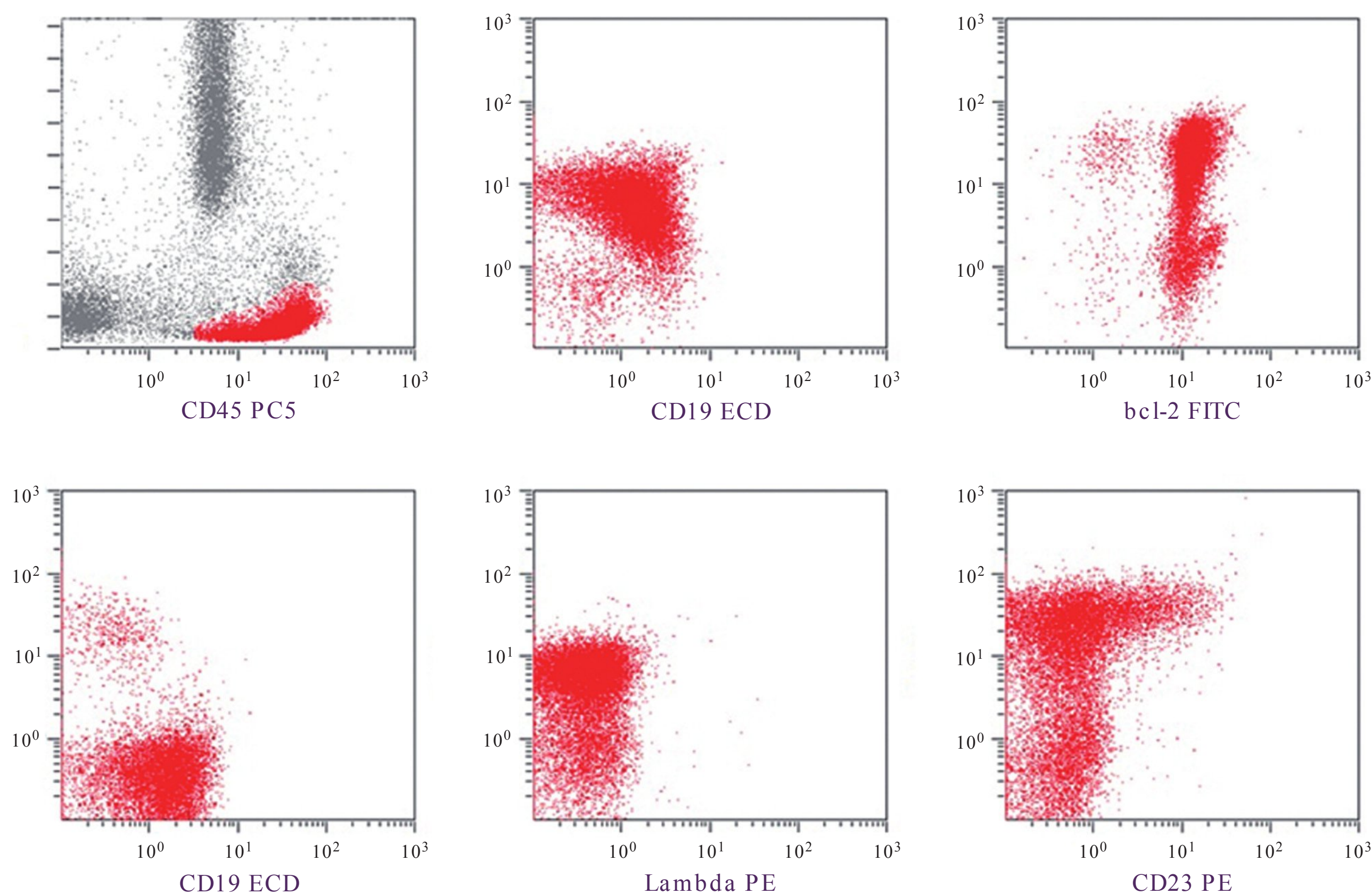


FIGURE 6.29.1 Flow cytometric histograms show dual staining of CD10 and CD19, as well as CD20 and bcl-2. A monoclonal k pattern, positive FMC-7, and partial positive CD23 are also demonstrated. A small percentage of T cells are also present in the gated population, as represented by the CD5-positive and bcl-2-positive/CD20-negative clusters. SS, side scatter; PC5, phycoerythrin–cyanin 5; PE, phycoerythrin; ECD, phycoerythrin–Texas Red; FITC, fluorescein isothiocyanate.

cleaved lymphocytes (centrocytes) and large and small noncleaved lymphocytes (centroblasts) as well as phagocytic macrophages. Polarization of the lymphoid cells is frequently demonstrated in the germinal centers in FH; small and large cleaved lymphoid cells are on one pole (light zone), and small and large noncleaved transformed lymphoid cells are on another pole (dark zone). In the dark zone, the mitotic rate is high and there are many tangibly macrophages, forming a “starry-sky” pattern.

TABLE 6.29.1

Characteristic Morphologic Features of FL

Histologic pattern	Presence of evenly distributed uniform follicles with incomplete or absent mantle zones and back-to-back pattern
Cytology	Predominantly centrocytes, mixed centrocytes and centroblasts, and predominantly centroblasts
Special features	Same as histologic pattern

In the Working Formulation, FL is divided into three subtypes: predominantly small-cleaved cell type (large cell <20%), mixed small-cleaved and large-cell type (large cell 20% to 50%), and predominantly large-cell type (large cell >50%) (5). The predominantly small-cleaved cell subtype is the most common form, accounting for 40% to 50% of all FL cases. The predominantly large-cell subtype is the least common among the three subtypes.

The International Lymphoma Study Group is of the opinion that these three subtypes are a continuous gradation in the number of large cells and are difficult to reproduce. Therefore, this group proposes these subtypes to be a grading system rather than subclassification (7). However, the grading system is still based on the proportion of cell components: grade I, small-cell predominant; grade II, mixed small and large cells; and grade III, large-cell predominant.

The WHO scheme adopts the Berard cell-counting method for grading. The calculation is based on the average number of centroblasts in 10 neoplastic follicles examined under a 40× high-power field (hpf) (9). Grade 1 FL is defined by the presence of 0 to 5 centroblasts/hpf (Fig. 6.29.2); grade 2, 6 to 15 centroblasts/hpf; and grade 3, >15 centroblasts/hpf (Fig. 6.29.3). Grade 3 is further divided into grades 3a and 3b. In the former, centrocytes

TABLE 6.29.2

Differentiation between Follicular Hyperplasia (FH) and Follicular Lymphoma (FL)

	FH	FL
Lymph node architecture	Well preserved	Completely or partially effaced
Size and shape of germinal center	Variable	Uniform
Distribution of follicles	Irregular, well separated	Even, back-to-back pattern
Density of follicles	Low	High
Margin of follicles	Sharp	Poorly defined
Mantle zone	Intact	Absent or incomplete
Infiltration of capsule	Absent or minimal	Present
Cells within germinal centers	Polymorphic	Monomorphic
Polarization in germinal centers	Present	Absent
Mitotic rate	High	Low
Tangible-body macrophages	Prominent	Rare
Cells in interfollicular area	Normal lymphocytes	Atypical lymphoid cells
Surface immunoglobulin	Polyclonal	Monoclonal
CD45RA (MT2)	Negative	Positive
CD10	Positive	Positive
bcl-2 protein	Negative	Positive
bcl-6 protein	Positive	Positive
% Ki-67	Low	High
t(14;18)	Absent	Present
Molecular biology	No oncogene	BCL-2
Immunoglobulin gene	Germline	Rearrangement

Modified from Sun T, Susin M. Differential Diagnosis of Lymphoid Disorders. New York: Igaku-Shoin; 1996:91, with permission.

are still present, whereas the latter shows solid sheets of centroblasts. The experts on the WHO classification panel consider that, because there are only minor differences in natural history and response to treatment between grade 1 and grade 2, their division is clinically insignificant. However, the three-grade system is retained in the WHO classification to avoid confusion (8).

The accuracy of this grading system depends on the clear distinction between centrocytes, centroblasts, and follicular dendritic cells. The characteristics of a centrocyte are mainly based on nuclear configuration, which may show prominent clefts, indentation, or linear infoldings. The nuclear chromatin is condensed, and the small nucleoli are inconspicuous. The cytoplasm is scant. The centroblasts are two to three times as large as normal lymphocytes. Their nuclei are usually round but occasionally irregular in configuration, with vesicular chromatin and one to three nucleoli, which are typically opposed to the nuclear membrane. Their cytoplasm is scant and is basophilic on Giemsa stain. The follicular dendritic cells show nuclei with a size similar to those in centroblasts and distinct eosinophilic nucleoli in a vesicular chromatin background. The cytoplasm is indistinct. However, the most

characteristic feature is the presence of double nuclei with flattening of the adjacent nuclear membranes (Fig. 6.29.4).

Diffuse area is frequently present in various proportions with the follicles. A diffuse area is defined as an area lacking follicular structure but is positive for follicular dendritic cell stains (CD21+/CD23+). A follicular pattern is defined by the presence of >75% of follicular area. A follicular and diffuse pattern should be reported when the follicular area is between 25% and 75%. When follicular area is <25%, it is designated as focally follicular/predominantly diffuse (10). The grade and percentages of follicular and diffuse areas should be included in the diagnosis. Sclerosis may be present in diffuse areas, particularly in cases after therapy, forming a nodular sclerotic pattern (Fig. 6.29.5). The association of sclerosis with prognosis is controversial (11).

If the diffuse area is composed of mainly centrocytes, it is not considered to be clinically significant (10). When the diffuse area is composed of predominantly centroblasts, it should be diagnosed as diffuse large B-cell lymphoma (Fig. 6.29.6). When the lymph node shows a completely diffuse pattern and comprises predominantly centrocytes with a minor component of centroblasts, it may represent a diffuse FL. However, such a diagnosis should be always

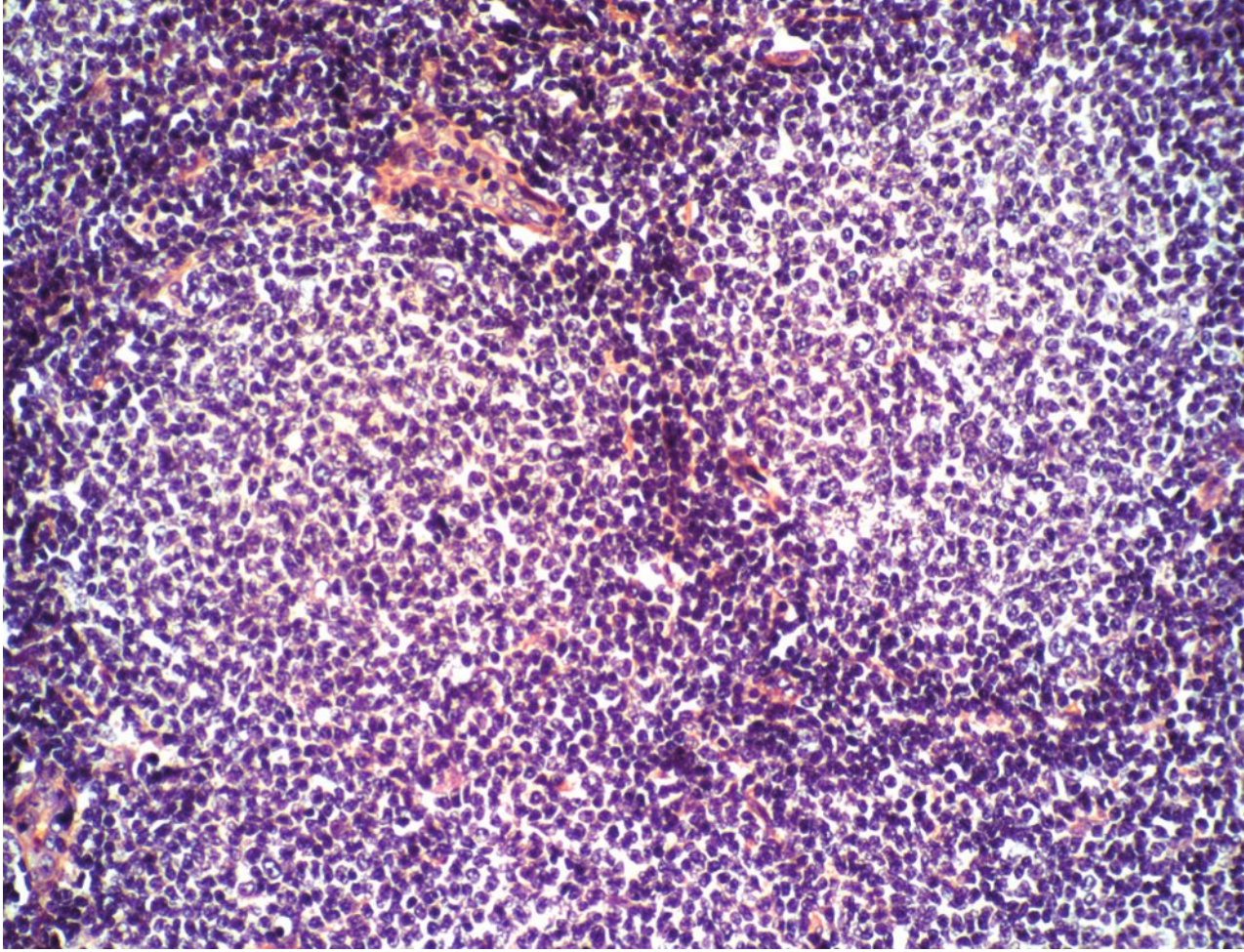


FIGURE 6.29.2 Lymph node biopsy shows a grade 1 FL composed predominantly of centrocytes. The two malignant follicles appear similar in size and shape with a back-to-back pattern. Hematoxylin and eosin, 20× magnification.

confirmed with immunophenotyping and/or molecular genetic studies (10). If the neoplastic component is predominantly centroblasts, the diagnosis should be diffuse large B-cell lymphoma.

There are several morphologic variants of FL. The presence of extrafollicular monocytoid B cells is considered a predictor of worse prognosis with shorter failure-free survival and overall survival (14). The marginal zone variant or “reverse” variant is seen in about 10% of FL cases (9,15) (Fig. 6.29.7). Other rare variants include the signet-ring cell variant, the floral variant, and the variant with amorphous extracellular precipitate (9,15). The 2008 WHO classification designates three clinical variants: pediatric FL, primary intestinal FL, and other extranodal FL. However,

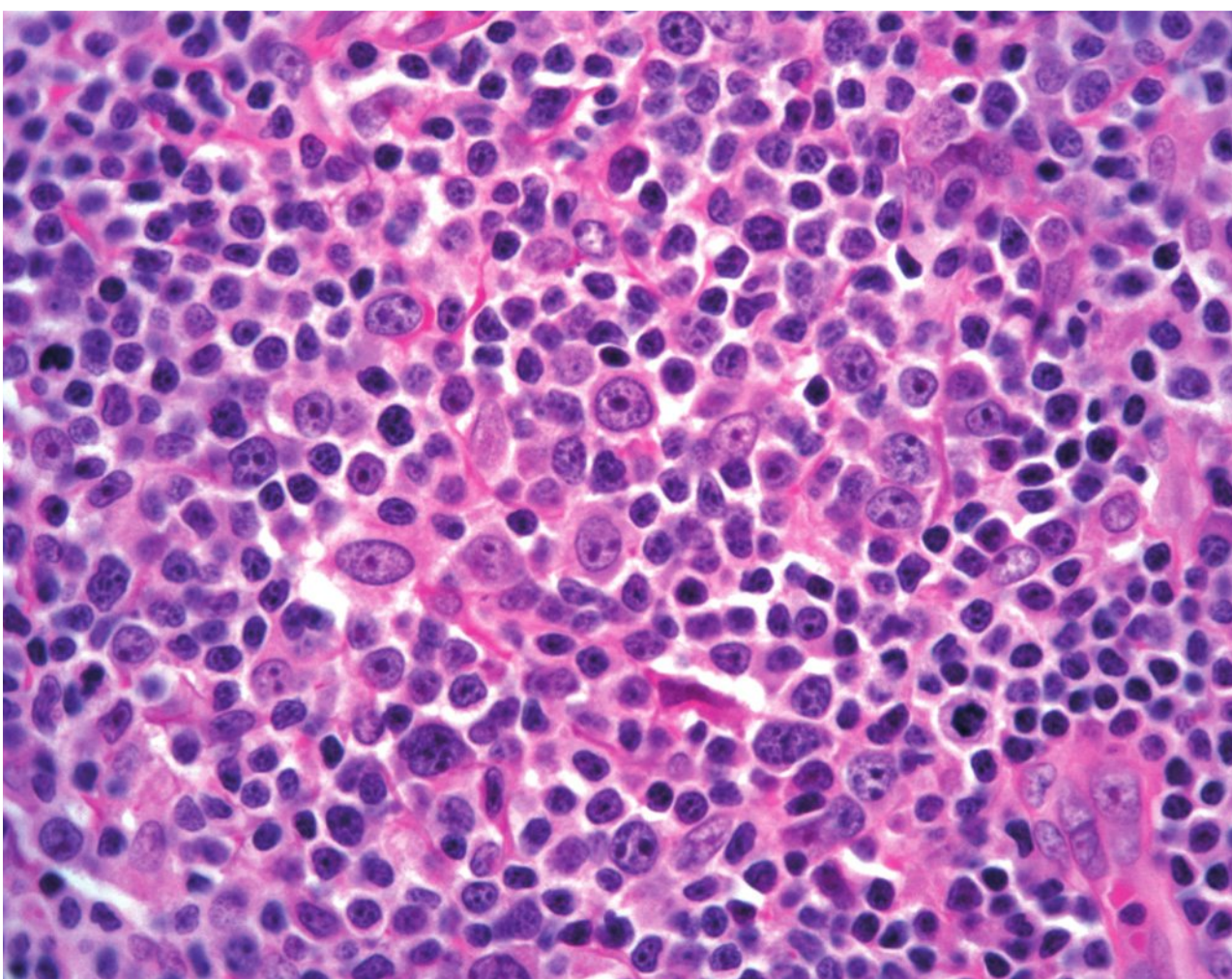


FIGURE 6.29.3 Lymph node biopsy reveals a grade 2 FL with several centroblasts present in the center of the field. Hematoxylin and eosin, 60× magnification.

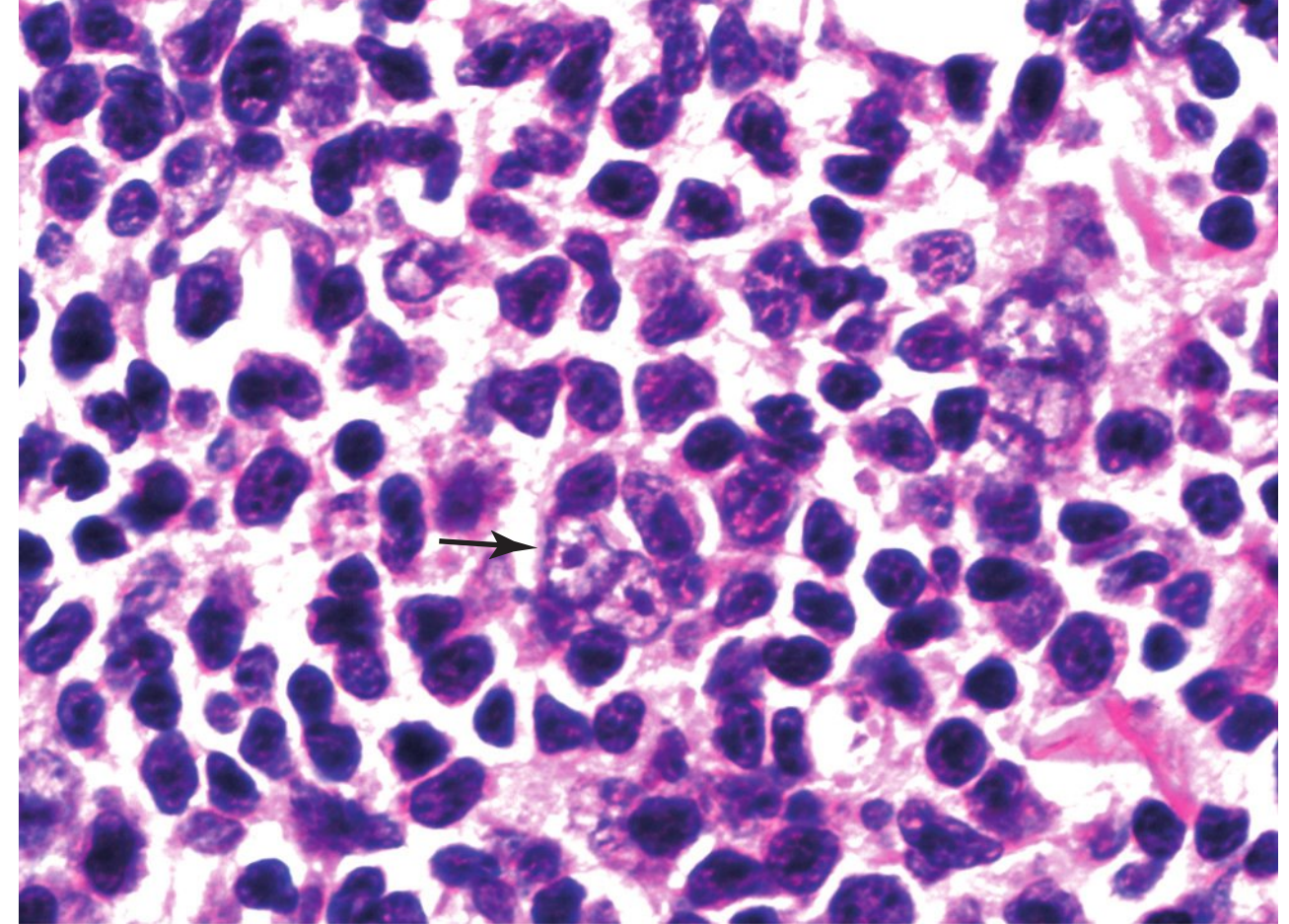


FIGURE 6.29.4 Lymph node biopsy shows a pair of follicular dendritic cells in the center (arrow). Note flattening of the adjacent nuclear membranes. There are a few large centrocytes above the follicular dendritic cells. These cells should be distinguished from the centroblasts. Hematoxylin and eosin, 100× magnification.

when skin is primarily involved, it is called primary cutaneous follicle center lymphoma (see Case 30) (10).

The “in situ” FL is a rare entity with unknown clinical significance (16). Its definition is overexpression of bcl-2 in the centrocytes and centroblasts of one or more follicles in a normal histologic background. As bcl-2 can be present in T lymphocytes, primary follicles and mantle zones, the staining of these cells should not be interpreted as in situ FL.

The initial diagnosis of FL should not be made in the extranodal sites, because a nodular lymphoid aggregate in an extranodal tissue is not necessarily an indication of FL. In contrast, a follicular pattern may not be present in the extranodal tissue even if it is an FL. In the bone marrow, FL is characterized by the presence of a well-defined

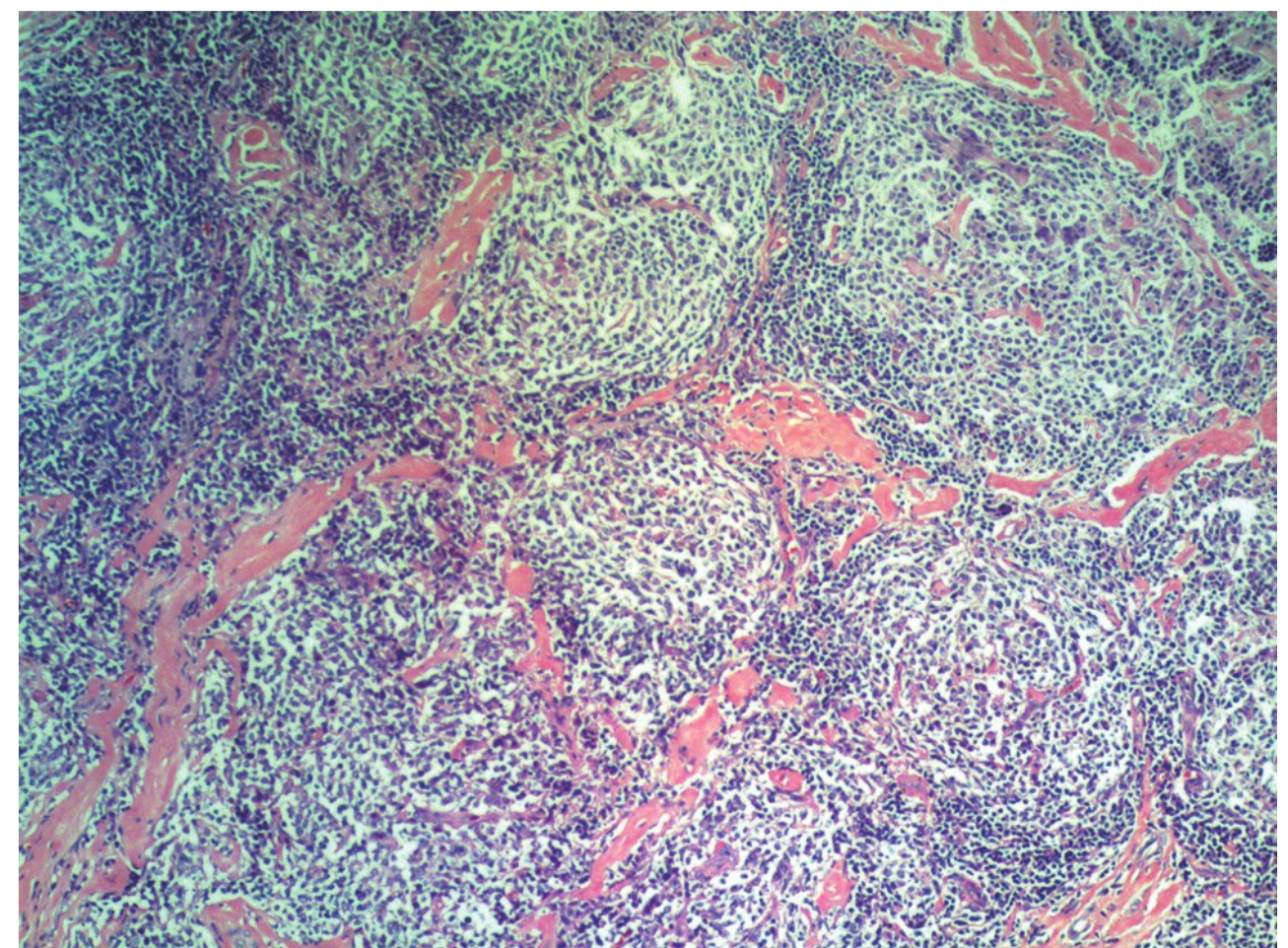


FIGURE 6.29.5 Lymph node biopsy shows broad hyaline fibrous bands surrounding the follicles, forming a nodular sclerosing pattern. Hematoxylin and eosin, 10× magnification.

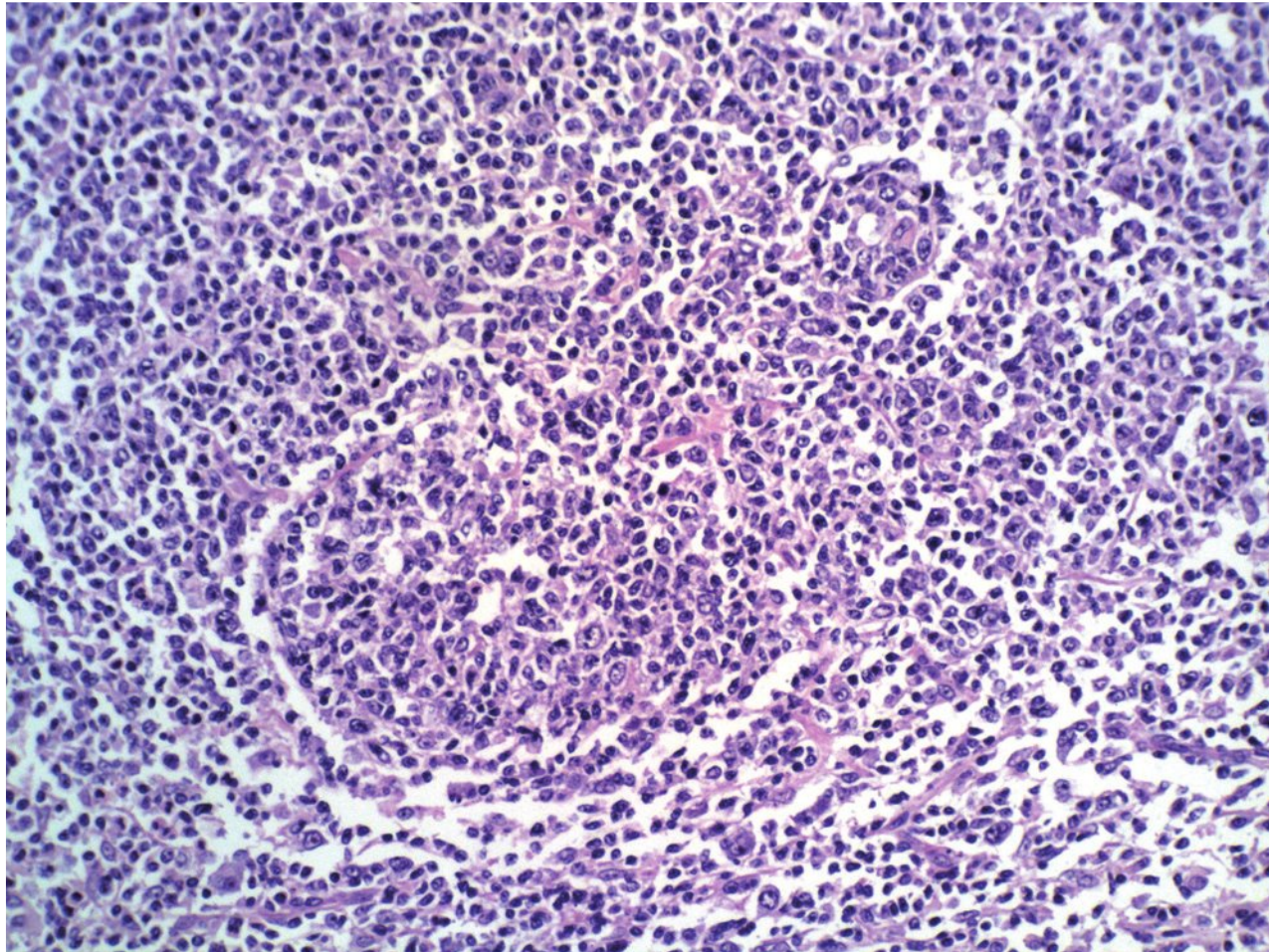


FIGURE 6.29.6 Lymph node biopsy reveals two residual follicles surrounded by diffuse large lymphoma cells, representing transformation to diffuse large B-cell lymphoma. Hematoxylin and eosin, 20× magnification.

paratrabeular lymphoid aggregate rather than a nodular aggregate in the intertrabeular area (Figs. 6.29.8 and 6.29.9) (7,11). The cytologic features may be identical to those of the nodal tumor, but tumor cells in the bone marrow often appear more mature than those in the lymph node. Multiple large paratrabeular lymphoid follicles containing atypical lymphoid cells with infiltration of the surrounding normal bone marrow are features in favor of malignancy.

In the spleen, evenly distributed, uniform, white nodules may be present on the cut surface in cases of FL. Microscopically, these nodules are located in the lymphoid follicles of the splenic white pulp. In the large-cell subtype, the tumor nodules may have an irregular shape, and their distribution may be uneven (11). The distinction between FL and FH in the spleen can be very difficult if cytologic atypia is not obvious.

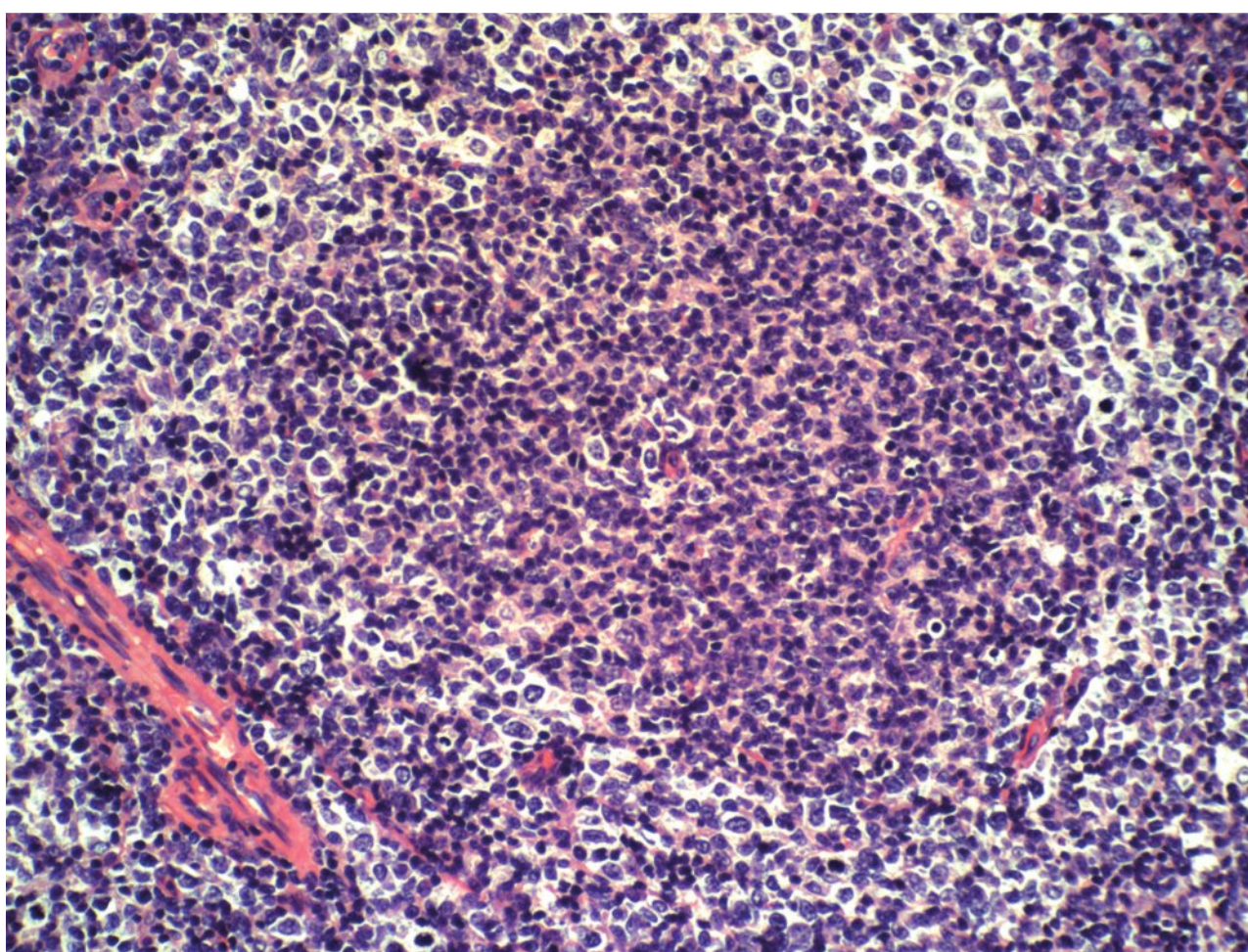


FIGURE 6.29.7 Lymph node biopsy reveals a marginal zone pattern in an FL. Hematoxylin and eosin, 20× magnification.

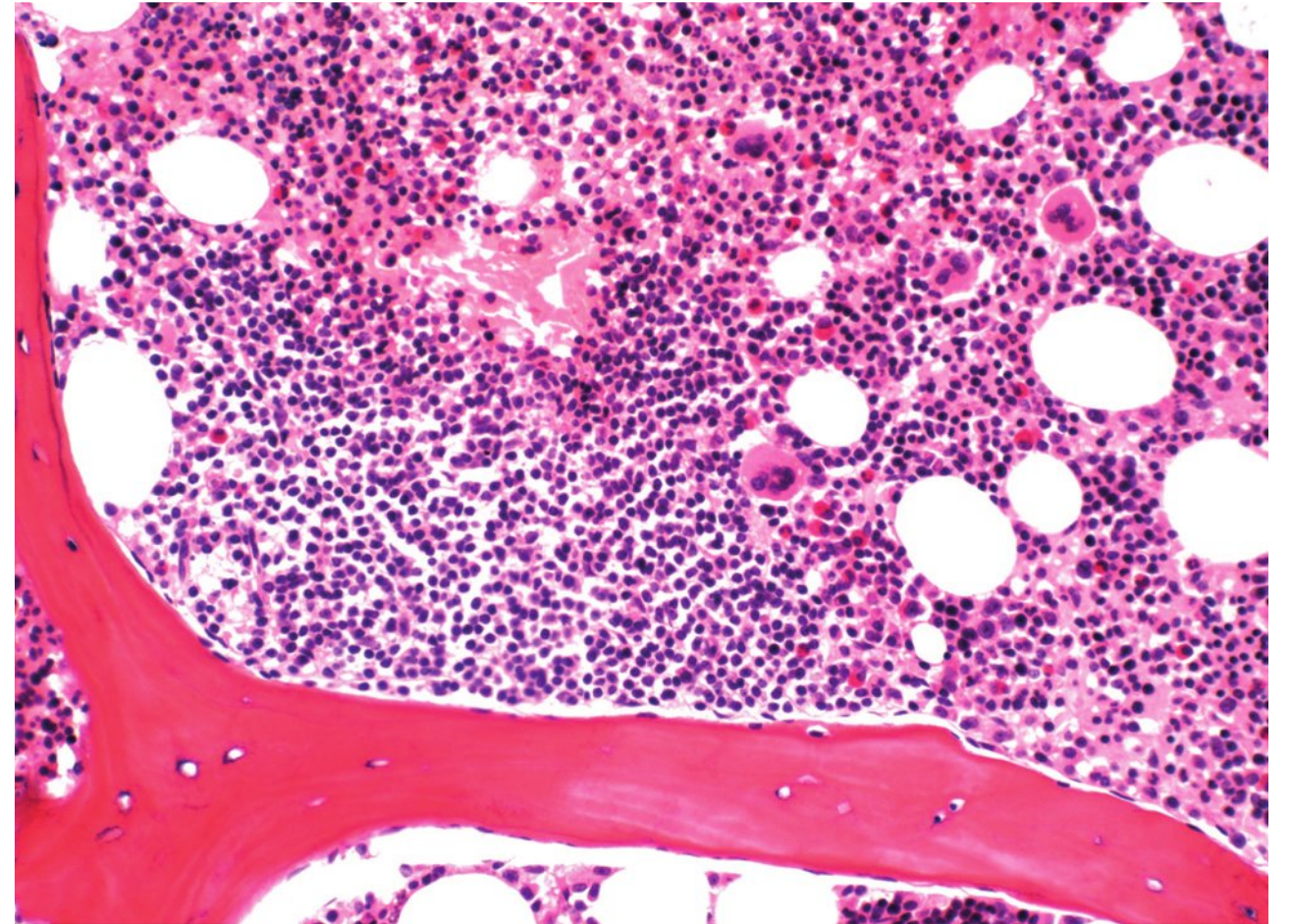


FIGURE 6.29.8 Bone marrow biopsy shows a paratrabeular lymphoid infiltration pattern, characteristic of FL. Hematoxylin and eosin, 20× magnification.

In the liver, FL involves primarily the portal tracts of the liver and may spread beyond the limiting plate of the lobules. The infiltration pattern is the same as other types of lymphomas. Its distinction from FH depends on cytologic atypia, hepatic parenchymal involvement, and the absence of plasma cells in the infiltrate (11).

Primary FL of the gastrointestinal tract is rare and accounts for <7% of all non-Hodgkin lymphomas at that location (17). The recent development of wireless capsule endoscopy and double-balloon endoscopy has helped detect increasing numbers of FL not only in the small intestine but also in other parts of the gastrointestinal tract, including the esophagus, stomach, colon, and rectum (18). Multiple lymphomatous polyposis was initially considered to be a typical feature of mantle cell lymphoma, but has recently been found in a large number of gastrointestinal FL with an incidence ranging from 15.0% to 46.2% (18,19).

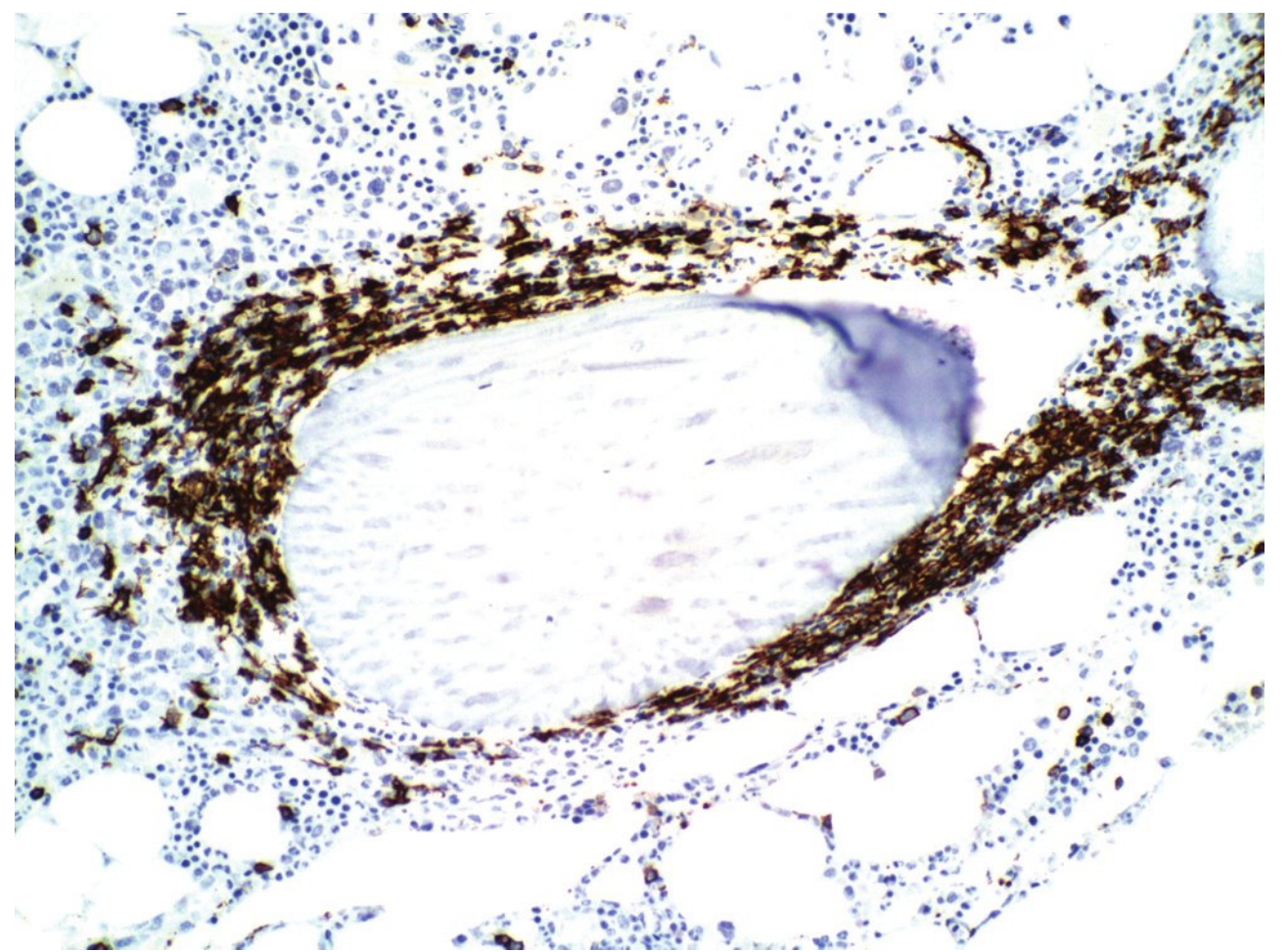


FIGURE 6.29.9 Bone marrow biopsy shows a paratrabeular lymphoid infiltration pattern with positive CD20 staining. Immunoperoxidase, 20× magnification.

FL cells are relatively frequently demonstrated in the blood. The identification of these tumor cells depends on the demonstration of a monoclonal B-cell population with a positive CD10 antigen. The presence of characteristic lymphocytes with cleft nucleus detected by light or electron microscopy may also help to make a specific diagnosis of FL (Fig. 6.29.10) (20).

FL has the tendency to transform into high-grade lymphomas. The transformation rate varies from 10% to 80% in different reports, depending on the follow-up period, frequency of biopsies, and inclusion of autopsy data (21–23). Most cases show transformation into diffuse large B-cell lymphoma (21). It may also transform into CD30-positive anaplastic large-cell lymphoma (21) or blastic and/or blastoid form (23) on rare occasions.

By using fine-needle aspirates, the diagnosis of FL is based on the presence of lymphoid aggregates, two-nuclei-like cleaved cells, irregular nuclei, and nucleoli (24). In a good aspirate, grading can be attempted by estimation of the percentages of centroblasts (25). For instance, centroblasts are <10% in grade 1, averaging 25% in grade 2, and >50% in grade 3. However, morphology alone is usually not sufficient to make a definitive diagnosis; flow cytometry, immunocytochemistry, or fluorescence in situ hybridization is frequently needed to substantiate the diagnosis (24,25).

Immunophenotype

A large monoclonal antibody panel can be used for immunophenotyping of FL. All the common B-cell-associated antigens (CD19, CD20, CD22, CD24, and CD79a) are present in FL (1,9,26). The specific antigens for FL are CD10, bcl-2, and bcl-6. In addition, the follicular dendritic cell markers (CD21, CD23, CD35) can demonstrate the dendritic meshwork among the tumor cells and are useful to substantiate the diagnosis of FL (3,9). Follicular dendritic cells may provide a favorable microenvironment for FL in extranodal sites such as the bone marrow (27) and may play an important role in the prognosis of the patients (28). IRF4/MUM1 is typically absent in FL (10).

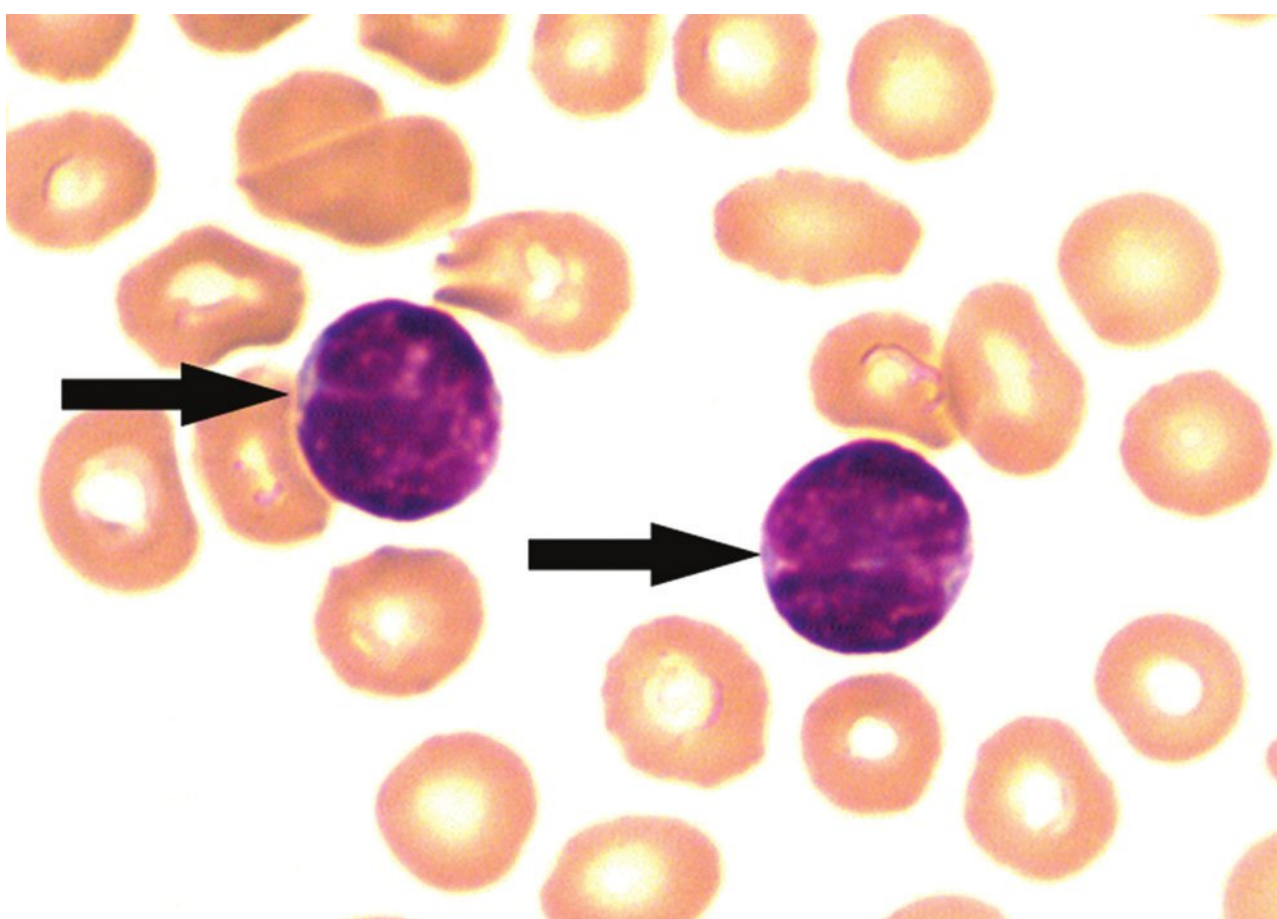


FIGURE 6.29.10 Peripheral blood smear reveals two cleaved small lymphocytes (arrows). Wright–Giemsa, 200× magnification.

The immunophenotype of FL reflects its germinal center cell origin, which is shared by Burkitt lymphoma and a subset of diffuse large B-cell lymphoma. Burkitt lymphoma, however, usually shows an absence of bcl-2 (9,29). The diffuse large B-cell lymphoma with this immunophenotype can be the result of transformation from FL (30).

Mantle cell lymphoma can be bcl-2 positive, with the presence of a dendritic meshwork, but it is CD5+, CD10–, bcl-6–, CD43+ (1,26). The dendritic meshwork, as demonstrated by CD21 and CD23 staining, in FL is arranged in a nodular spherical pattern, but the meshwork in mantle cell lymphoma shows a loose pattern (31). FL is usually negative for CD43, but a small subset of FL expresses CD43 staining. In those cases, the tumor cells were predominantly large-cell type with focally diffuse areas (32).

FH can be easily distinguished from FL by the lack of monoclonality. It is also negative for bcl-2 and CD45RA (9,33). A new set of monoclonal antibodies to kappa and lambda light chains may also help to distinguish FH from FL by clonality identification (34).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry can demonstrate a monoclonal B-cell population with a positive reaction to CD10 and bcl-2 in FL, but bcl-2 can also be demonstrated by flow cytometry in cases of FH; thus, it is not a reliable marker. However, immunohistochemical staining has the advantage in recognizing the follicular pattern with the special staining of CD10 (Fig. 6.29.11), bcl-2 (Fig. 6.29.12), and bcl-6 (Fig. 6.29.13) identified in the follicular structure. It is important that the staining is not outside the malignant follicles unless there are extensive diffuse areas. Bcl-2 can cross-react with inter-follicular T cells; such cross-reaction should not be counted as positive reaction. The ability of demonstrating the follicular dendritic meshwork by CD21 and CD23 (Fig. 6.29.14) stains is another advantage of immunohistochemistry.

The percentage of Ki-67–positive cells (proliferation index [PI]) (Fig. 6.29.15) generally correlates with the FL

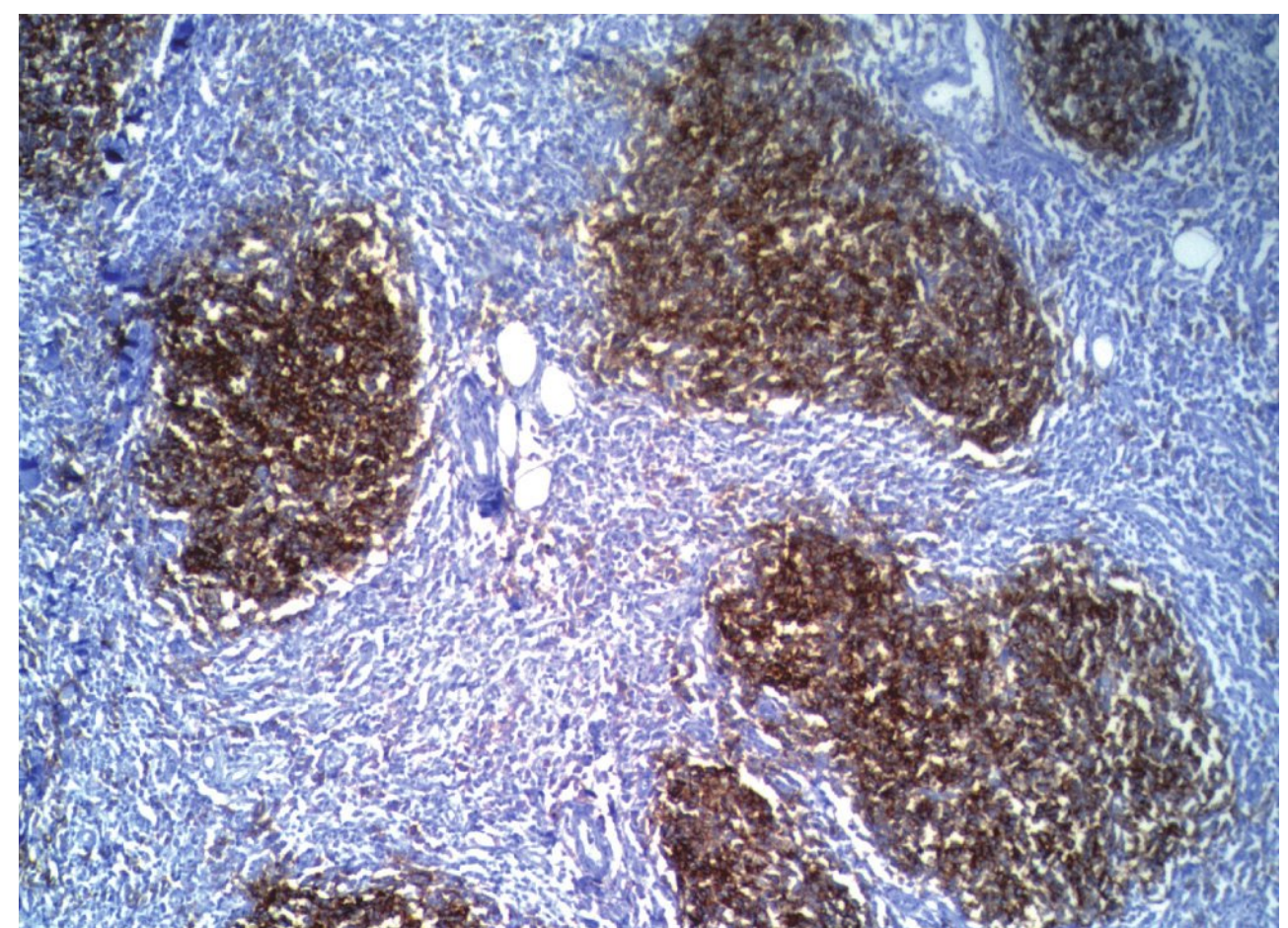


FIGURE 6.29.11 Lymph node biopsy shows positive CD10 staining in malignant follicles. Immunoperoxidase, 10× magnification.

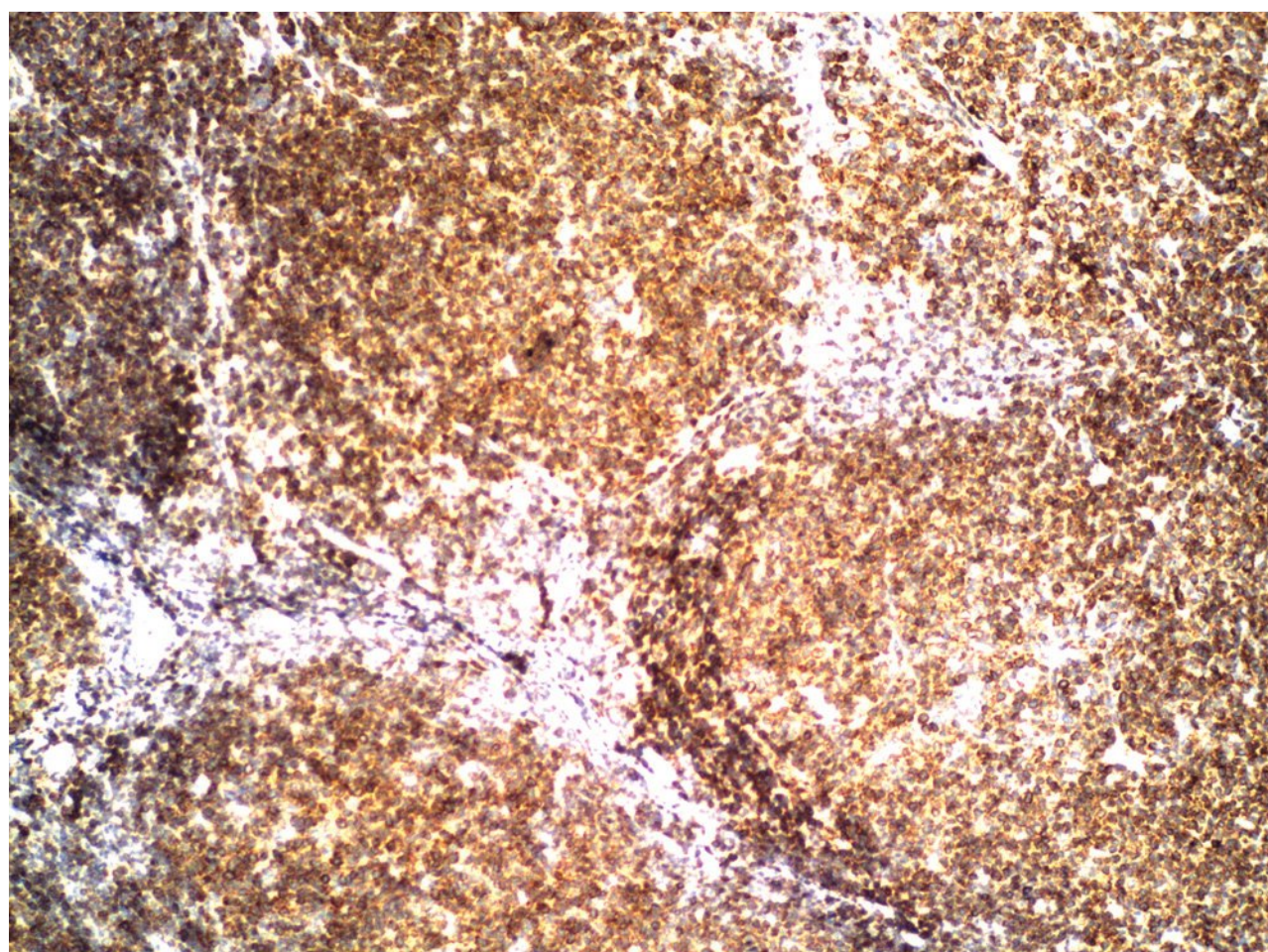


FIGURE 6.29.12 Lymph node biopsy shows positive bcl-2 staining in malignant follicles. Immunoperoxidase, 10× magnification.

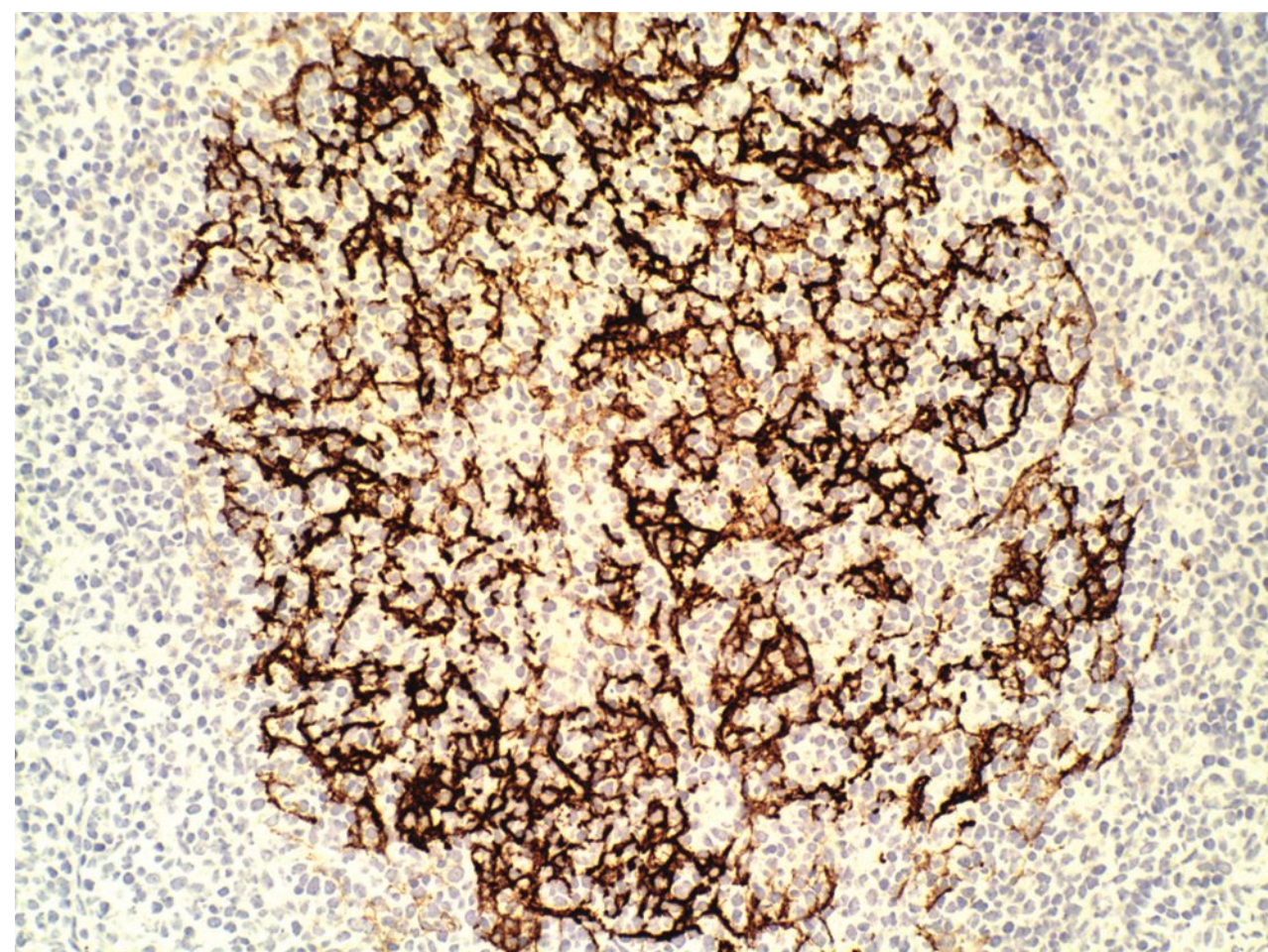


FIGURE 6.29.14 Lymph node biopsy shows a dendritic meshwork in the malignant follicle demonstrated by CD23 staining. Immunoperoxidase, 20× magnification.

grades. However, there is a subset of low-grade FL that shows a high PI (35). These low-grade, high-PI cases behave clinically like grade 3 FL. It has been found recently that the S-phase kinase-associated protein 2 (SKP2), an inhibitor of p27, can also help grading and predict transformation of FL (36).

In the current case, the patient had a typical clinical presentation of multiple lymphadenopathies and a waxing and waning clinical course. Histologically, the lymph node showed a typical follicular pattern, and the malignant follicles showed positive CD20, bcl-2, and bcl-6, but were negative for CD3 and CD5. Flow cytometry showed a similar phenotype, that is, positive CD19, CD10, and bcl-2 but negative CD5. Therefore, the diagnosis of FL was established. The bone marrow in this case showed paratrabecular lymphoid infiltrates with a flow cytometric phenotype similar to that of the lymph node, indicating a stage 4 disease.

The second lymph node biopsy revealed large-cell infiltration in the interfollicular area representing transformation to a diffuse large B-cell lymphoma.

Molecular Genetics

FL is characterized by t(14;18)(q32;q21) translocation (Fig. 6.29.16). The heavy-chain gene is located at 14q32, whereas the protooncogene, bcl-2, is located at 18q21. When bcl-2 moves into the proximity of the immunoglobulin (Ig) heavy-chain gene enhancer region, it becomes deregulated or activated, and the functional bcl-2-Ig fusion protein is overexpressed. The bcl-2 gene encodes for an inner mitochondrial membrane protein that plays the role of blocking programmed cell death (apoptosis) (37). Therefore, cells with abnormal expression of this protein remain in stage G₀ in the cell cycle and become immortalized (1,11). Because the protein does not promote

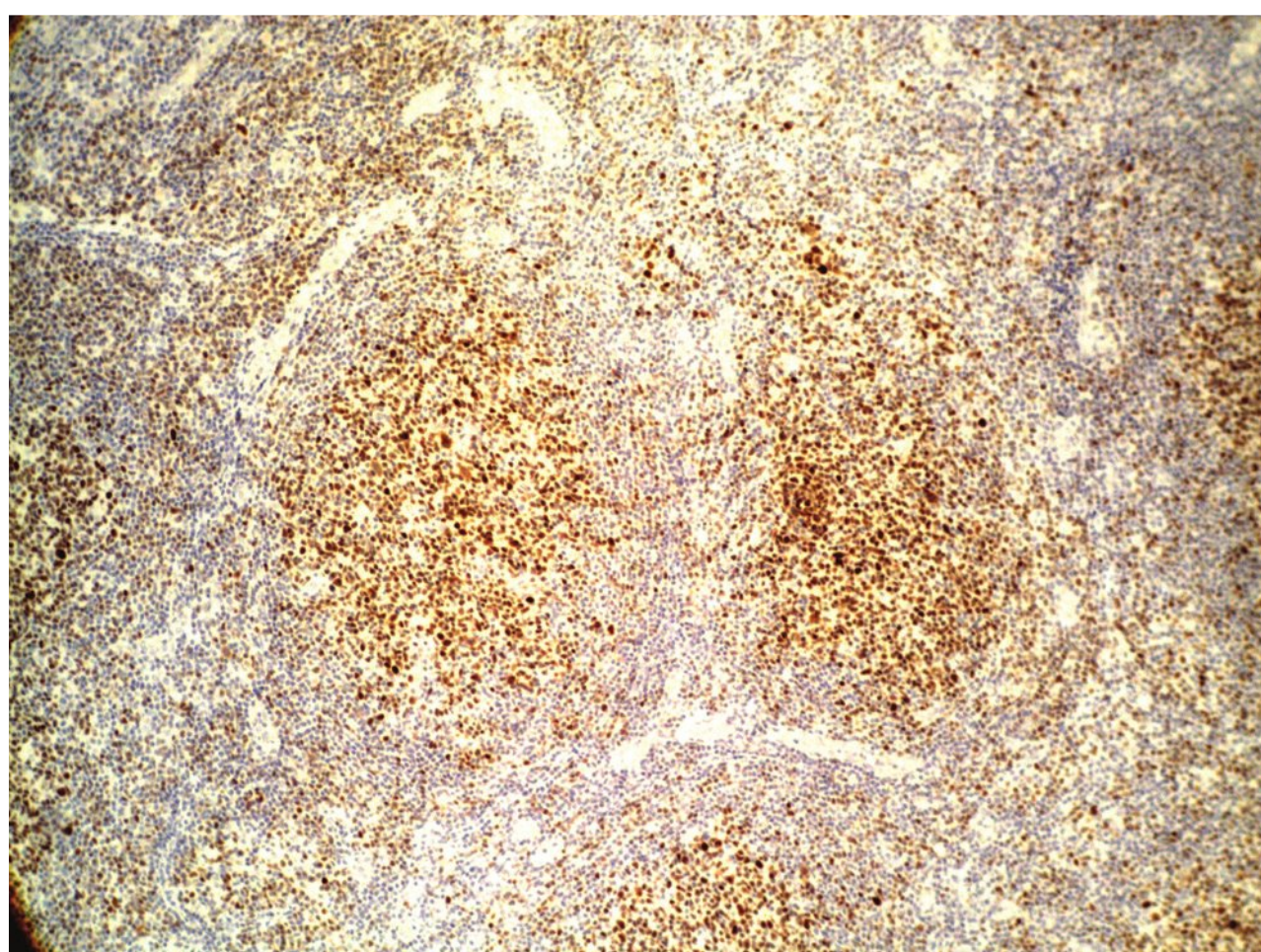


FIGURE 6.29.13 Lymph node biopsy shows positive bcl-6 staining in malignant follicles. Immunoperoxidase, 10× magnification.

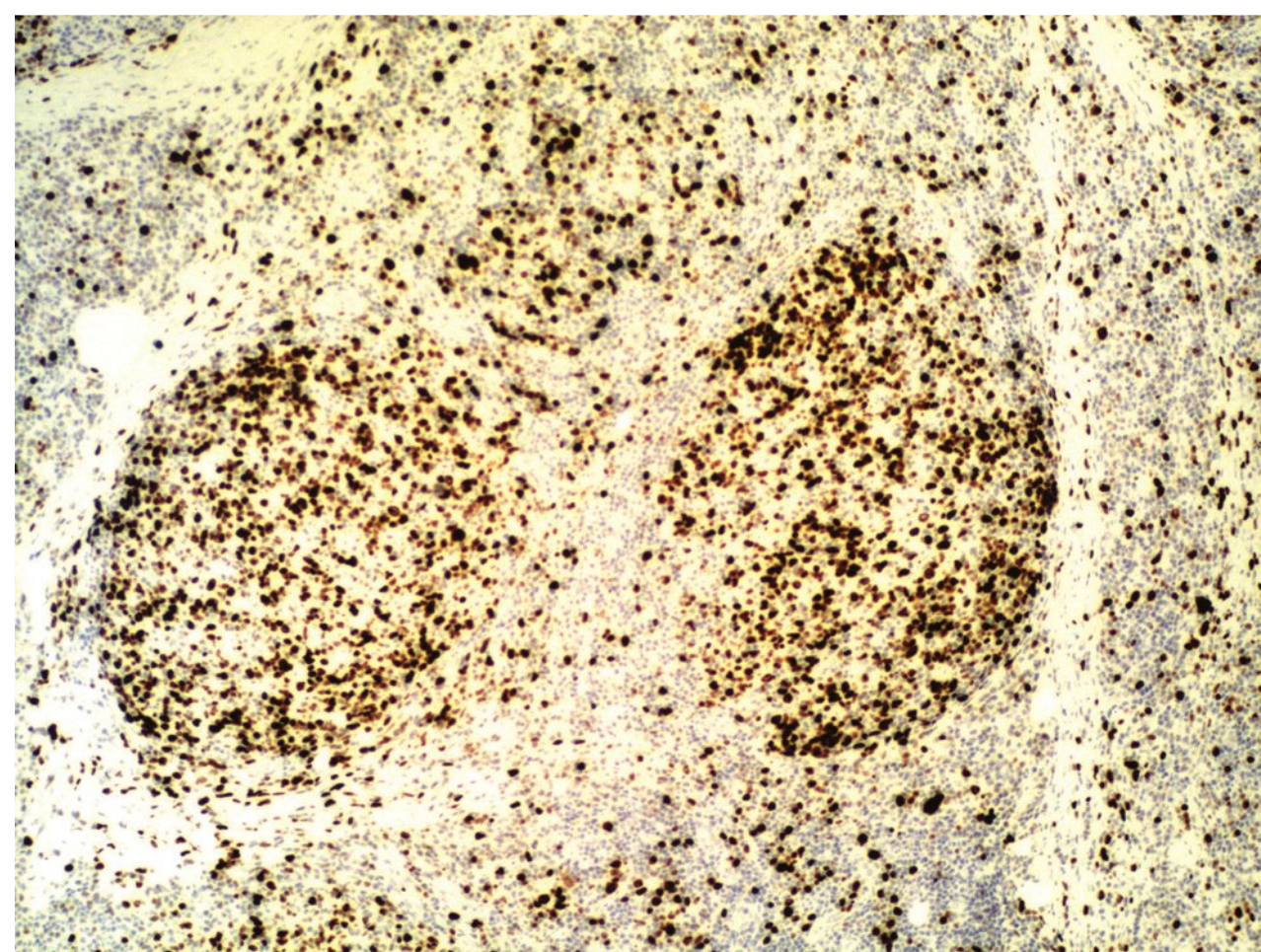


FIGURE 6.29.15 Lymph node biopsy shows moderate Ki-67 staining in a malignant follicle. Immunoperoxidase, 10× magnification.

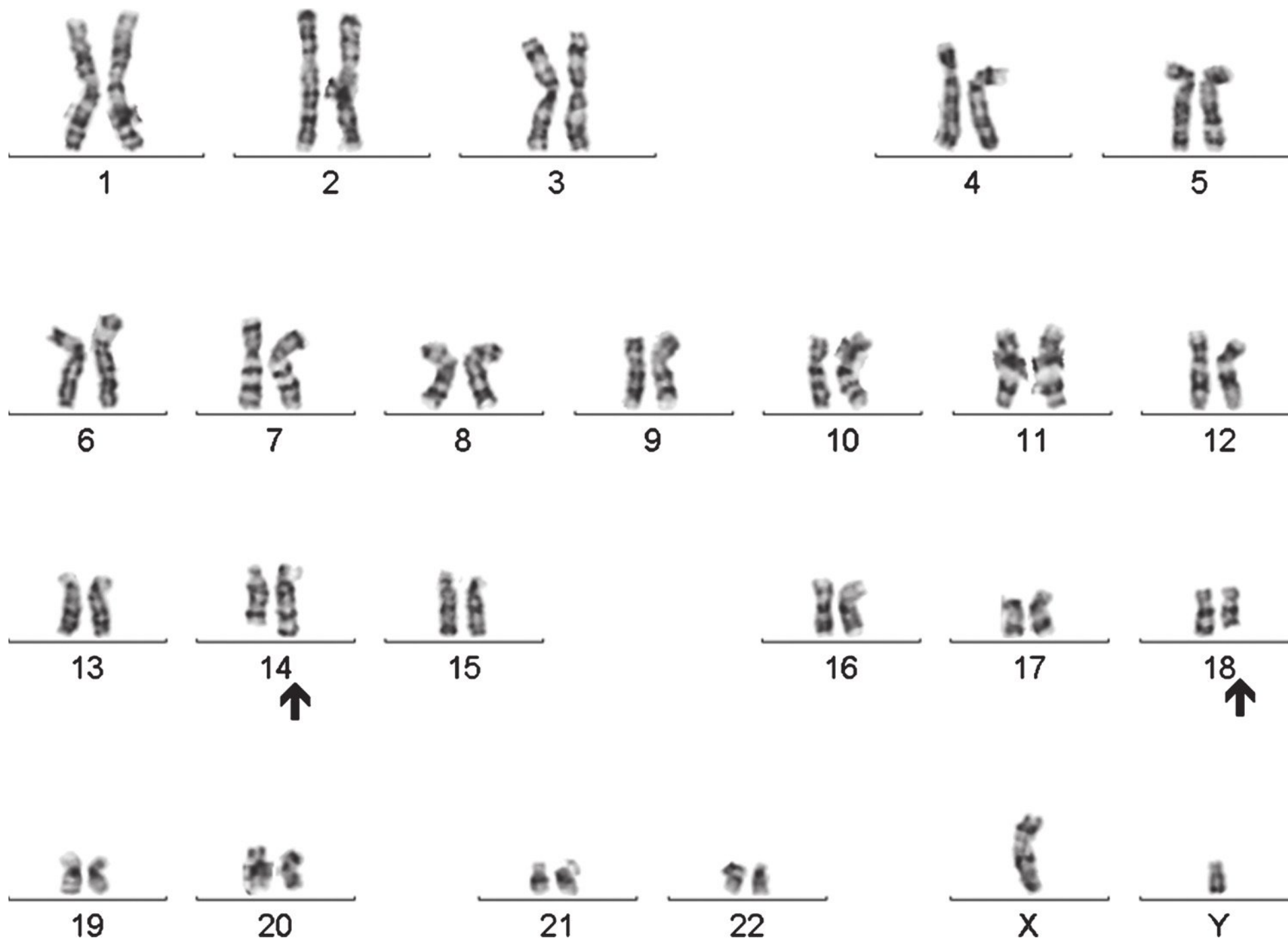


FIGURE 6.29.16 A karyotype of lymph node cells shows chromosomal $t(14;18)(q32;q21)$ translocation. (Original source: Quest Diagnostics Nichols Institute).

proliferation, it serves to explain why most patients with FL have an indolent clinical course.

Studies with transgenic mice show that $t(14;18)$ translocation alone does not induce tumorigenesis. A second oncogene, such as the *MYC* gene, or other chromosomal abnormality is required for neoplastic transformation (26). Furthermore, $t(14;18)$ is present in small clones of B cells in 30% to 50% of healthy individuals (26). Therefore, the results of using molecular techniques to detect $t(14;18)$ for the diagnosis of minimal residual disease should be interpreted with caution. In fact, circulating cells carrying the $t(14;18)$ translocation can be demonstrated in some FL patients even after a continuous complete remission of >10 years (26).

Generally, 80% to 90% of cases of FL in the United States and Europe express $t(14;18)$ (38). However, the frequency of this translocation is lower in Asia. In a study of Japanese patients, only 59.6% of FL cases showed $t(14;18)$ (4). In North America and Europe, the frequency of $t(14;18)$ in grade 3b FL is also low (39).

The variation in reported incidence also depends on the techniques used. Two studies have found cytogenetic analysis to be more sensitive (73% to 89% sensitivity), followed by Southern blotting (68% to 75%), with polymerase chain reaction being the least sensitive (49% to 65%) (40,41). Recent studies indicate that the fluorescence in situ hybridization technique is the most sensitive and has been used routinely in many laboratories (42,43).

This translocation can also be demonstrated in 30% of diffuse large B-cell lymphomas and occasionally in other lymphoproliferative disorders. The demonstration of *bcl-2* protein with dual B-cell antigen staining is reliable for the diagnosis of FL (44). However, *bcl-2* protein can be shown

in other B-cell lymphomas; in those cases, $t(14;18)$ may not be demonstrated (45).

Sole $t(14;18)$ aberration is only present in 10% of FL cases (9). The remaining cases may have additional breaks or additions. Common abnormalities include +7, +18, 3q27-28, 6q23-26, and 17p (9). In some FL cases with negative *bcl-2* gene rearrangement, *bcl-6* aberrations can be demonstrated (4). The rearrangement of *bcl-6* was found in about 15% of FL, and 5' mutations of the *bcl-6* gene are found in approximately 40% of FL (1). In a recent study, the *bcl-6* protein was demonstrated in all 31 grade 1 and 2 FL cases by immunohistochemistry and in all 5 FL cases by Western blotting (46). In the same study, *bcl-6* was positive in only 1 of 13 cases with small lymphocytic lymphomas, 1 of 12 mantle cell lymphomas, but none of 16 marginal zone lymphomas. In a study of FL with plasmacytic differentiation, both the lymphoma cells and plasma cells showed the same abnormalities in *BCL-2*, *BCL-6*, and *IgH@* gene rearrangements, indicating that the plasmacytic component is also neoplastic (47).

Another important question is cytogenetic evolution in the transformation of FL to diffuse large B-cell lymphoma. The involvement of single genes, such as *MYC*, *p53*, *CDKN2A/B*, and others, has been reported, but none of them can be held accountable for all sets of patients (48).

FL shows immunoglobulin heavy- and light-chain gene rearrangement with hypermutation in the variable region of the immunoglobulin heavy-chain gene (*Hv*). There is also intraclonal heterogeneity. These findings prove that FL is of follicle center cell origin.

The recent application of gene expression profiling is most promising in stratification of FL patients for predicting the clinical course and prognosis. An unexpected

TABLE 6.29.3

Salient Features for Laboratory Diagnosis of FL

1. Monoclonal surface immunoglobulin with bright fluorescence
2. Positive B-cell antigens: CD19, CD20, CD22, CD24, CD79a, HLA-DR
3. Characteristic markers for diagnosis: CD10, bcl-2, bcl-6
4. Additional markers: Ki-67 to demonstrate proliferation index (PI); CD21 and CD23 to demonstrate intrafollicular meshwork
5. Characteristic cytogenetics: t(14;18)(q32;q21)
6. Characteristic molecular biology: IgH/BCL-2
7. Immunoglobulin gene rearrangement with hypermutation in Hv region
8. BCL-6 gene rearrangement or 5' mutation

HLA-DR, human leukocyte antigen-DR; Ig, immunoglobulin; Hv, variable region of immunoglobulin heavy-chain gene.

finding has been reported by the National Cancer Institute group, which has identified two discrete gene expression profiles (28). The immune response 1 signature encodes genes expressed by T cells and macrophages and confers a favorable prognosis. The immune response 2 signature includes genes expressed predominantly by monocytes and follicular dendritic cells and confers an unfavorable prognosis. In this study, it appears that the clinical course is mainly influenced by the infiltrating nontumor cells and not the genes of the lymphoma cells.

A study with tissue microarray for immunohistochemical staining further supports this concept (49). This study demonstrated that CD4+ T cells and CD68+ macrophages were associated with poor prognosis, but programmed death 1 (PD-1)+, CD8+, and FOXP3 (defined regulatory T cell)-positive cells were associated with good prognosis. The CD4 cells may rescue the FL cells from apoptosis through the interaction between CD40 (on FL cells) and its legend, CD40L (on helper T cells) (50).

Another gene expression profiling study found that genes involved in cell-cycle regulation and DNA synthesis, including CXCL12, NEK2, and MAPK1, were differentially expressed in indolent and aggressive cases (51). The salient features for laboratory diagnosis of FL are summarized in Table 6.29.3.

Clinical Features

FL is a lymphoma of older age groups. It is seldom seen in patients younger than 20 years and almost never encountered in young children (1). It occurs with equal frequency in both sexes.

FL has an insidious onset. Therefore, when a diagnosis is made, the disease is already in an advanced stage: About 67% of patients are in stage III or IV at the time of diagnosis (2). Constitutional symptoms, such as fever, weight loss, and night sweats, are present in only 17% of patients. The

major clinical presentation is peripheral lymphadenopathy, involving mostly the cervical and inguinal lymph nodes (1,2). Extranodal involvement is rare but can be present in the spleen, Waldeyer ring, or gastrointestinal tract. Skin and soft tissue presentations are very rare: Bone and central nervous system have not been reported as primary sites. In advanced stages, however, bone marrow and liver are most commonly invaded (1). About 33% of patients show a leukemic blood picture (52), but circulating lymphoma cells have been demonstrated with polymerase chain reaction in 75% of patients who are in stages I and II (53).

Although patients with FL have an indolent clinical course with a medium survival of 9 to 10 years, FL is generally incurable (38). At the beginning, most patients may respond well to chemotherapy or radiation therapy, but the disease gradually becomes refractory to treatment with repeated relapses and finally leads to the death of the patient (1). The cause of death in most cases is due to transformation to high-grade lymphomas. Some patients may develop acute lymphoid or myeloid leukemia (1).

There have been many studies on the prognostic predictors. The International Prognostic Index identified advanced age, elevated serum LDH, poor performance status, advanced stage, and multiple extranodal sites as adverse prognostic features (54). The Follicular Lymphoma International Prognostic Index proposed five adverse prognostic factors, which include age ≥ 60 years, hemoglobin < 12 g/dL, LDH above normal range, stage III or IV disease, and the presence of >3 nodal sites (55).

The detection of BCL-2 gene rearrangement in patients after treatment is a reliable marker to predict prognosis (56). Further study of the breakpoint site in tumor cells shows that cases with a breakpoint at the minor cluster region have a better 3-year failure-free survival than do cases with a breakpoint at the major breakpoint region (57). However, if there is no BCL-2 rearrangement at the initial diagnosis, these cases have the worst prognosis than the other two groups (57). As mentioned in the Molecular Genetics section, gene expression profiling is a promising technique for stratification of FL cases to guide the treatment and predict the prognosis.

REFERENCES

1. Harris N, Ferry JA. Follicular lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:805–822.
2. Feller AC, Diebold J. *Histopathology of Nodal and Extranodal Non-Hodgkin's Lymphomas* (based on the WHO classification). 3rd ed. Berlin: Springer; 2004:53–66.
3. de Jong D. Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. *J Clin Oncol*. 2005;23:6358–6363.
4. Sekiguchi N, Kobayashi Y, Yokota Y, et al. Follicular lymphoma subgrouping by fluorescence in situ hybridization analysis. *Cancer Sci*. 2005;96:77–82.
5. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas. *Cancer*. 1982;49:2112–2135.

6. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas. *Lancet*. 1988;1:292–293.
7. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
8. Harris NL, Jaffe ES, Diebold J, et al. Lymphoma classification—from controversy to consensus: the R.E.A.L. and WHO classification of lymphoid neoplasms. *Ann Oncol*. 2000;11(suppl 1):S3–S10.
9. Nathwani BN, Harris NL, Weisenburger D, et al. Follicular lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:162–167.
10. Harris NL, Swerdlow SH, Jaffe ES, et al. Follicular lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO classification of tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:220–228.
11. Mann RB. Follicular lymphomas. In: Jaffe E, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. Philadelphia, PA: W.B. Saunders; 1995:252–282.
12. Nathwani BN, Winberg CD, Diamond LW, et al. Morphologic criteria for the differentiation of follicular lymphoma from florid reactive follicular hyperplasia: a study of 80 cases. *Cancer*. 1981;48:1794–1806.
13. Schnitzer B. The reactive lymphadenopathies. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:537–568.
14. Nathwani BN, Anderson JR, Armitage JO, et al. Clinical significance of follicular lymphoma with monocytoid B cells. *Hum Pathol*. 1999;30:263–268.
15. Sun T, Susin M. *Differential Diagnosis of Lymphoid Disorders*. New York: Igaku-Shoin; 1996:90–101.
16. Cong P, Basso S, Zecca M, et al. In situ localization of follicular lymphoma: description and analysis by laser capture microdissection. *Blood*. 2002;99:3376–3382.
17. Damaj G, Verkarre V, Delmer A, et al. Primary follicular lymphoma of the gastrointestinal tract: a study of 25 cases and a literature review. *Ann Oncol*. 2003;14:623–629.
18. Yamamoto S, Nakase H, Yamashita K, et al. Gastrointestinal follicular lymphoma: review of the literature. *J Gastroenterol*. 2010;45:370–388.
19. Kodama T, Obshima K, Nomura K, et al. Lymphomatous polyposis of the gastrointestinal tract, including mantle cell lymphoma, follicular lymphoma and mucosa-associated lymphoid tissue lymphoma. *Histopathology*. 2005;47:467–478.
20. Melo JV, Robinson DSF, Catovsky D. The differential diagnosis between chronic lymphocytic leukemia and other B-cell lymphoproliferative disorders. Morphological and immunological studies. In: Pollick A, Catovsky D, eds. *Chronic Lymphocytic Leukemia*. Churg, Switzerland: Harwood Academic Publishers; 1988:85–103.
21. Alsabeh R, Medeiros LJ, Glackin C, et al. Transformation of follicular lymphoma into CD30-large cell lymphoma with anaplastic cytologic features. *Am J Surg Pathol*. 1997;21:528–536.
22. Bastion Y, Sebban C, Berger F, et al. Incidence, predictive factors, and outcome of lymphoma transformation in follicular lymphoma patients. *J Clin Oncol*. 1997;15:1587–1594.
23. Natkunam Y, Warnke RA, Zehnder JL, et al. Blastic/blastoid transformation of follicular lymphoma. Immunohistologic and molecular analyses of five cases. *Am J Surg Pathol*. 2000;24:525–534.
24. Kishimoto K, Kitamura T, Fujita K, et al. Cytologic differential diagnosis of follicular lymphoma grades 1 and 2 from reactive follicular hyperplasia: cytologic features of fine-needle aspiration smears with Pap stain and fluorescence in situ hybridization analysis to detect t(14;18)(q32;q21) chromosomal translocation. *Diagn Cytopathol*. 2005;34:11–17.
25. Young NA. Grading follicular lymphoma on fine-needle aspiration specimens—a practical approach. *Cancer (Cancer Cytopathol)*. 2006;108:1–9.
26. Weisenburger DD, Chan WC. Lymphomas of follicles. Mantle cell and follicle center cell lymphomas. *Am J Clin Pathol*. 1993;99:409–420.
27. Bognar A, Csernus B, Bodor C, et al. Clonal selection in the bone marrow involvement of follicular lymphoma. *Leukemia*. 2005;19:1656–1662.
28. Dave SS, Wright G, Tan B, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor infiltrating immune cells. *N Engl J Med*. 2004;351:2159–2169.
29. Frost M, Newell J, Lones MA, et al. Comparative immunohistochemical analysis of pediatric Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Clin Pathol*. 2004;121:384–392.
30. Bertram HC, Check IJ, Milano MA. Immunophenotyping large B-cell lymphomas: flow cytometric pitfalls and pathologic correlation. *Am J Clin Pathol*. 2001;116:191–203.
31. Gloghini A, Carbone A. The non-lymphoid microenvironment of reactive follicles and lymphomas of follicular origin as defined by immunohistology on paraffin-embedded tissues. *Hum Pathol*. 1993;24:67–76.
32. Lai R, Weiss LM, Chang KL, et al. Frequency of CD43 expression in non-Hodgkin's lymphoma. A survey of 742 cases and further characterization of rare CD43+ follicular lymphomas. *Am J Clin Pathol*. 1999;111:488–494.
33. Utz GL, Swerdlow SH. Distinction of follicular hyperplasia from follicular lymphoma in B5-fixed tissues. Comparison of MT2 and bcl-2 antibodies. *Hum Pathol*. 1993;24:1155–1158.
34. Weiss LM, Loera S, Bacchi CE. Immunoglobulin light chain immunohistochemistry revisited, with emphasis on reactive follicular hyperplasia versus follicular lymphoma. *Appl Immunohistochem Mol Morphol*. 2010;18:199–205.
35. Wang SA, Wang L, Hochberg EP, et al. Low histologic grade follicular lymphoma with high proliferation index: morphologic and clinical features. *Am J Surg Pathol*. 2005;29:1490–1496.
36. Chiarle R, Fan Y, Piva R, et al. S-phase kinase-associated protein 2 expression in non-Hodgkin's lymphoma inversely correlates with p27 expression and defines cells in S phase. *Am J Pathol*. 2002;160:1457–1466.
37. Hockenberry D, Nunez G, Milliman C, et al. Bcl-2 is an inner-mitochondrial membrane protein that blocks programmed cell death. *Nature*. 1990;348:334–336.
38. Knutsen T. Cytogenetic mechanisms in the pathogenesis and progression of follicular lymphoma. *Cancer Surv*. 1997;30:163–192.
39. Ott G, Katzenberger T, Lohr A, et al. Cytomorphologic, immunohistochemical, and cytogenetic profiles of follicular lymphoma: 2 types of follicular lymphoma grade 3. *Blood*. 2002;99:3806–3812.
40. Horsman DE, Gascoyne RD, Coupland RW, et al. Comparison of cytogenetic analysis, Southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. *Am J Clin Pathol*. 1995;103:472–478.
41. Turner GE, Ross FM, Krajewski AS. Detection of t(14;18) in British follicular lymphoma using cytogenetics, Southern blotting and the polymerase chain reaction. *Br J Haematol*. 1995;89:223–225.
42. Poetsch M, Weber-Mathiesen K, Plendl HJ, et al. Detection of the t(14;18) chromosomal translocation by interphase cytogenetics with yeast-artificial-chromosome probes in follicular lymphoma and nonneoplastic lymphoproliferation. *J Clin Oncol*. 1996;14:963–969.

43. Einerson RR, Kurtin PJ, Dayharsh GA, et al. FISH is superior to PCR in detecting t(14;18)(q32;q21)-IgH/bcl-2 in follicular lymphoma using paraffin-embedded tissue samples. *Am J Clin Pathol*. 2005;124:421–429.
44. Cornfield DB, Mitchell DM, Almasri NM, et al. Follicular lymphoma can be distinguished from benign follicular hyperplasia by flow cytometry using simultaneous staining of cytoplasmic bcl-2 and cell surface CD20. *Am J Clin Pathol*. 2000;114:258–263.
45. Pezzella F, Tse AGD, Cordell JL, et al. Expression of the bcl-2 oncogene protein is not specific for 14;18 chromosomal translocation. *Am J Pathol*. 1990;137:225–232.
46. Raible MD, Hsi ED, Alkan S. Bcl-6 protein expression by follicle center lymphomas. A marker for differentiating follicle center lymphomas from other low grade lymphoproliferative disorders. *Am J Clin Pathol*. 1999;121:101–107.
47. Gradowski JF, Jaffe ES, Warnke RA, et al. Follicular lymphomas with plasmacytic differentiation include two subtypes. *Mod Pathol*. 2010;23:71–79.
48. Sigal S, Ninette A, Rechavi G. Microarray studies of prognostic stratification and transformation of follicular lymphomas. *Best Pract Res Clin Haematol*. 2005;18:143–156.
49. Wahlin BE, Aggarwal M, Montes-Moreno S, et al. A unifying microenvironment model in follicular lymphoma: Outcome is predicted by programmed death-1-positive, regulatory, cytotoxic, and helper T cells and macrophages. *Clin Cancer Res*. 2010;16:637–650.
50. Carbone A, Glohini A, Cabras A, et al. The germinal centre-derived lymphomas seen through their cellular microenvironment. *Br J Haematol*. 2009;145:468–480.
51. Glas AM, Kersten MJ, Delahaye LJ, et al. Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. *Blood*. 2005;105:301–307.
52. Elenitoba-Johnson KSJ, Gascoyne RD, Lim MS, et al. Homozygous deletions at chromosome 9q21 involving p16 and p15 are associated with histologic progression in follicle center lymphoma. *Blood*. 1993;82:2510–2516.
53. Lambrechts AC, Hupkes PE, Dorssers LCJ, et al. Translocation (14;18)-positive cells are present in the circulation of the majority of patients with localized (stage I and II) follicular non-Hodgkin's lymphoma. *Blood*. 1993;82:2510–2516.
54. A predictive model for aggressive non-Hodgkin's lymphoma. The International Non-Hodgkin's Lymphoma Prognostic Factors Project. *N Engl J Med*. 1993;329:987–994.
55. Solal-Celigny P, Roy P, Clombat P, et al. Follicular lymphoma international prognostic index. *Blood*. 2004;104:1258–1265.
56. López-Guillermo A, Cabanillas F, McLaughlin P, et al. The clinical significance of molecular response in indolent follicular lymphomas. *Blood*. 1999;93:3081–3087.
57. López-Guillermo A, Cabanillas F, McDonnell TI, et al. Correlation of bcl-2 rearrangement with clinical characteristic and outcome in indolent follicular lymphoma. *Blood*. 1999;93:3081–3087.

CASE 30

Primary Cutaneous Follicle Center Lymphoma

CASE HISTORY

A 63-year-old man presented with a 6-month history of skin growth on left upper forehead. The lesion was tender with no bleeding. The patient noticed that the mass grew and receded periodically, but the size remained the same. He denied having systemic symptoms, such as fever, night sweats, and weight loss. Physical examination of the lesion showed a 1 cm × 5 mm ill-defined and slightly violaceous dermal plaque on the left upper forehead. No cervical, axillary, or inguinal lymphadenopathy was detected. The spleen and liver were not palpable. The laboratory findings, including lactate dehydrogenase, liver function tests, creatinine, and erythrocyte sedimentation rate, were all within normal limits. A PET/CT scan was negative. A skin biopsy of the lesion showed lymphoma (Figs. 6.30.1 and 6.30.2). Bone marrow examination revealed no lymphoma involvement. The patient was treated with local radiation therapy. One year later, relapsed lymphomatous lesion appeared on the right upper forehead and was treated with local radiation again. The patient was doing well in a 3-year follow-up.

IMMUNOHISTOCHEMISTRY

Skin biopsy showed that the tumor cells were positive for CD20 (Fig. 6.30.3), and BCL-6 (Fig. 6.30.4), but were negative for BCL-2 (Fig. 6.30.5), CD3 (Fig. 6.30.6), CD5, and CD43. CD10 was weakly reactive to tumor cells. CD21 stain demonstrated a follicular dendritic meshwork in the tumor nodules.

MOLECULAR GENETICS

The specimen showed no immunoglobulin heavy-chain gene and T-cell receptor beta- and gamma-chain gene rearrangements. Polymerase chain reaction (PCR) analysis with the primers to the major and minor breakpoint cluster regions (MBR and mcr) did not show t(14;18).

DISCUSSION

Primary cutaneous follicle center lymphoma (PCFCL) is a clinicopathologic entity distinct from the nodal follicular

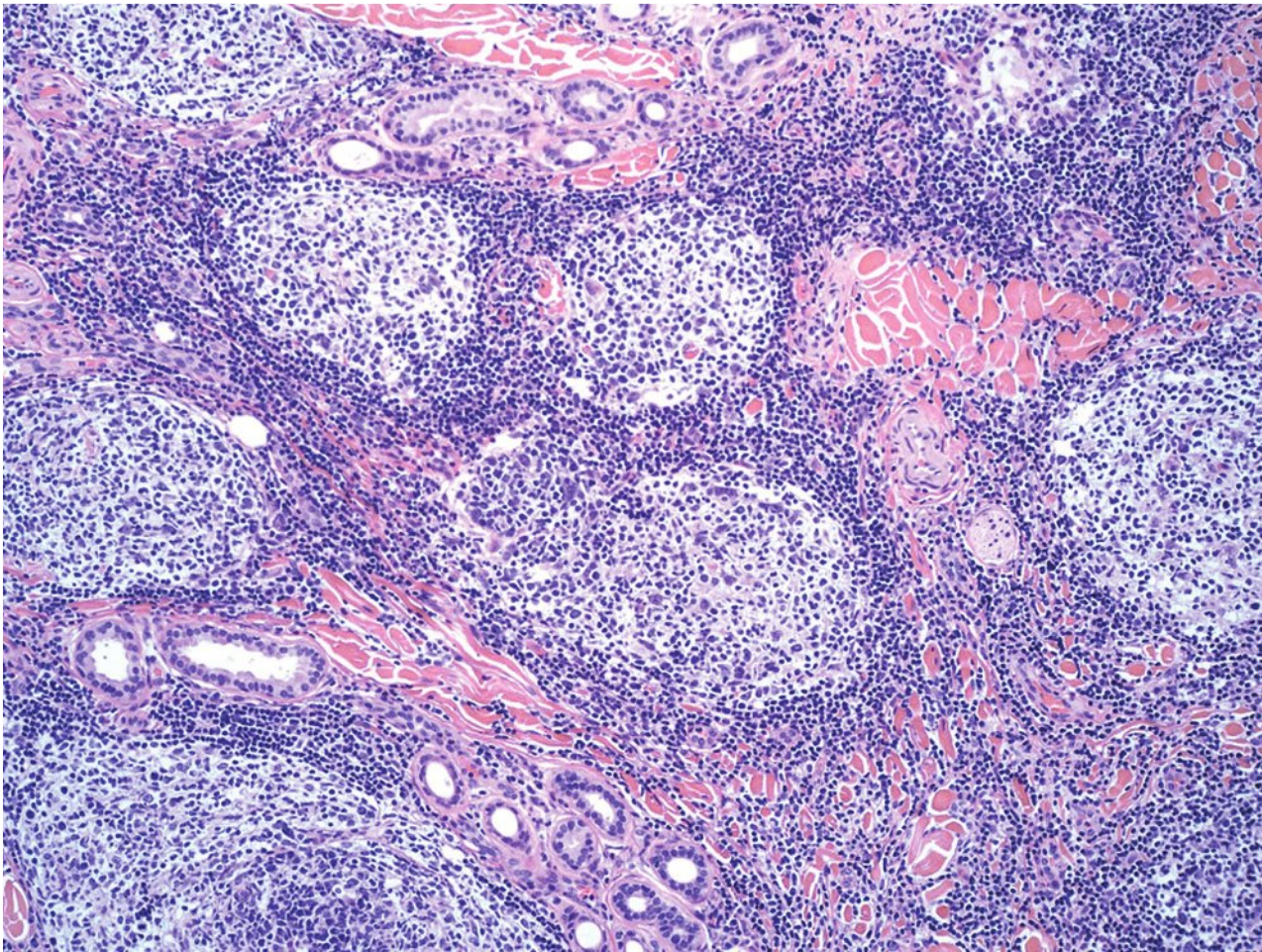


FIGURE 6.30.1 Skin biopsy shows multiple follicles surrounded by a collar of small lymphocytes mimicking a mantle zone. H&E, ×10.

lymphoma in terms of clinical presentation, immunophenotyping, molecular genetics, and prognosis. Before the advent of immunophenotyping and molecular genetics, this tumor was frequently mislabeled as pseudolymphoma. In 1951, Crosti reported seven cases and designated this tumor as reticulo-histiocytoma of the back, which was later called “Crosti lymphoma” (1). In 1997, the European Organization for Research and Treatment of Cancer (EORTC) classification of cutaneous lymphoma designated this neoplasm as primary cutaneous follicle center cell lymphoma (PCFCL) (2). The definition of PCFCL is based mainly on cytologic features with no specific criteria of histologic pattern and immunophenotyping. Therefore, it is actually a heterogeneous group of lymphoma, including diffuse large B-cell lymphoma and marginal zone B-cell lymphoma. In the World Health Organization (WHO)/EORTC classification, three main types of cutaneous B-cell lymphomas are recognized: cutaneous marginal zone

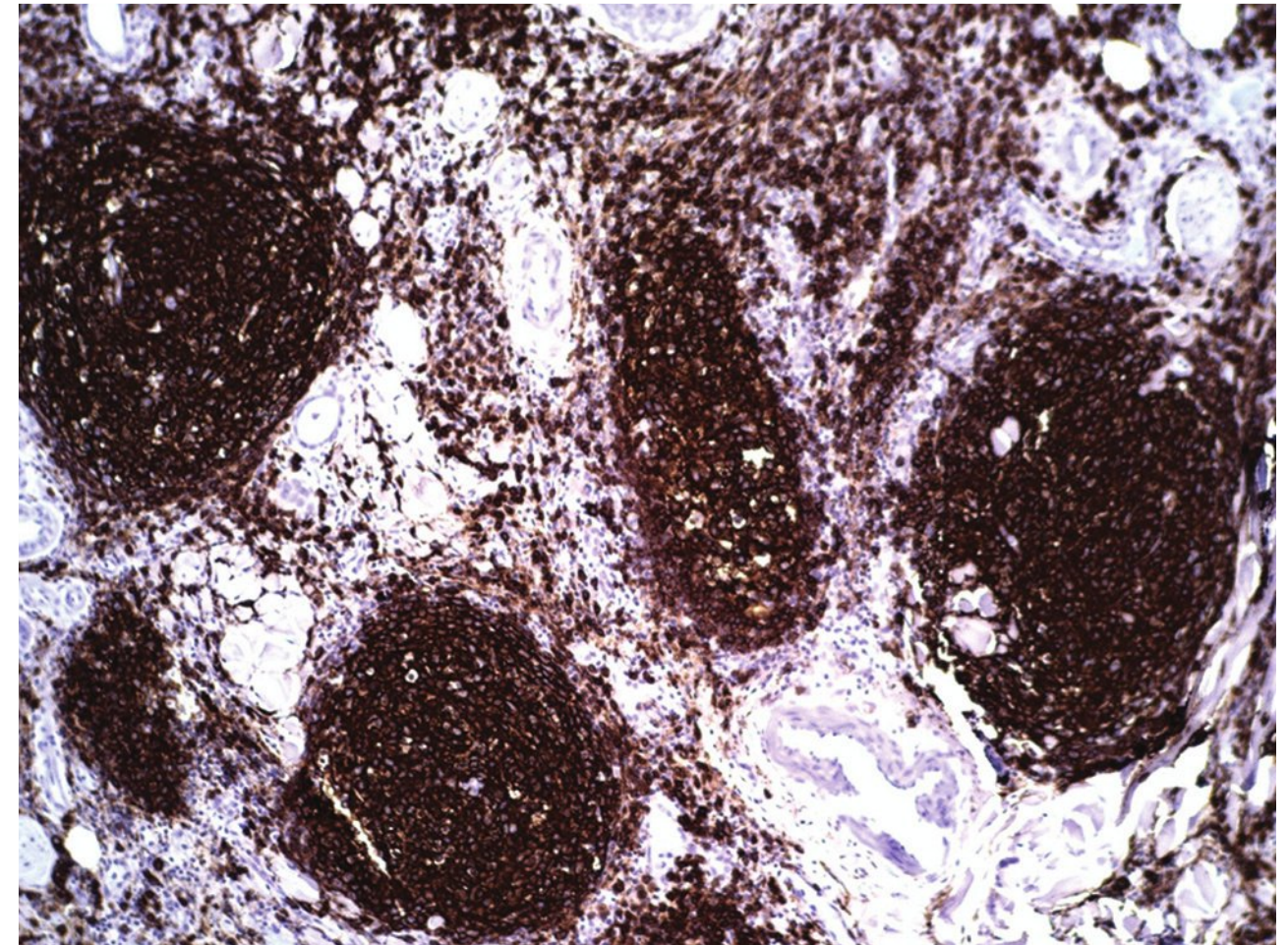


FIGURE 6.30.3 The follicles are positive for CD20 stains. Immunoperoxidase, ×10.

B-cell lymphoma, PCFCL, and cutaneous diffuse large B-cell lymphoma (3). The 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues adopts the new term, PCFCL (4).

Morphology

PCFCL has the highest incidence (60%) among primary cutaneous B-cell lymphomas. The lesion frequently involves the deep dermis, but some studies reveal no top or bottom heavy distribution (5). The epidermis is usually not involved. The diagnosis of PCFCL requires the presence of centrocytes and centroblasts. Unlike nodal follicular lymphoma, grading is not prognostically relevant in PCFCL (3). In advanced cases, the centrocytes are large, intermixed with multilobated cells and in occasional cases, spindle cells (4). The histologic pattern is variable and represents a histologic spectrum from follicular, follicular and diffuse to diffuse growth pattern. A lesion with a follicular pattern may

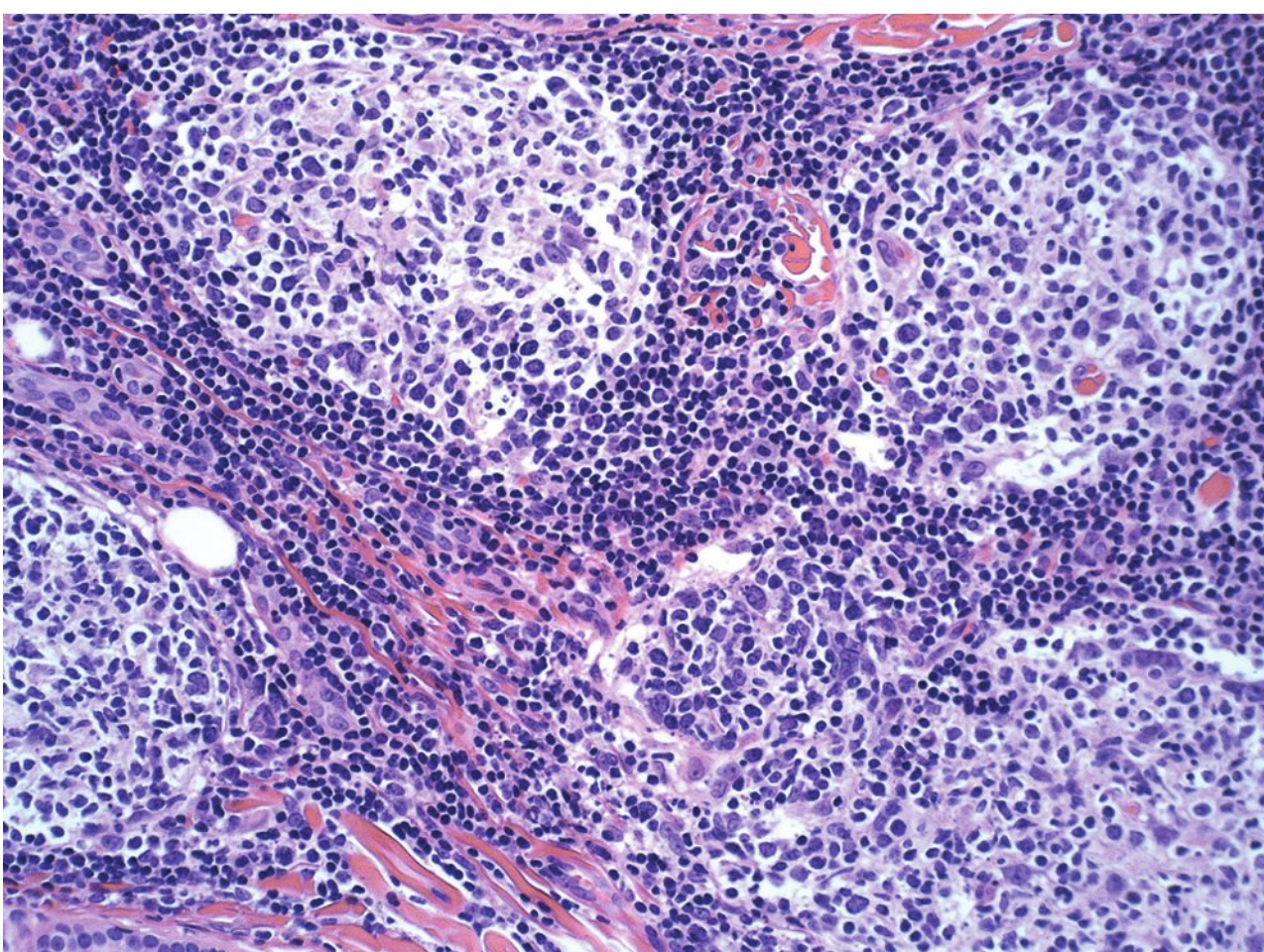


FIGURE 6.30.2 Higher magnification shows mixed population of centrocytes and centroblasts in the follicles. H&E, ×20.

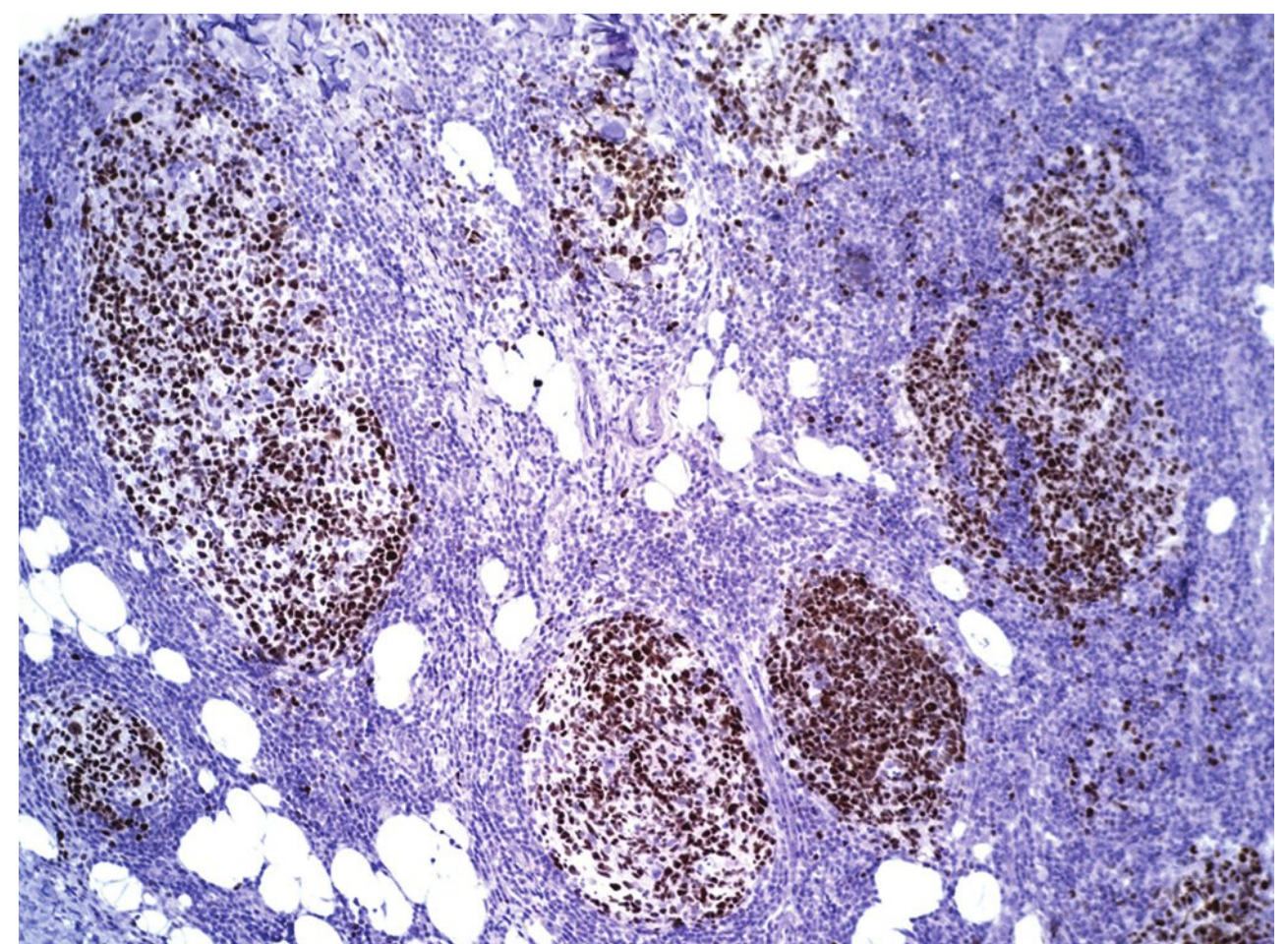


FIGURE 6.30.4 The follicles are positive for BCL-6. Immunoperoxidase, ×10.

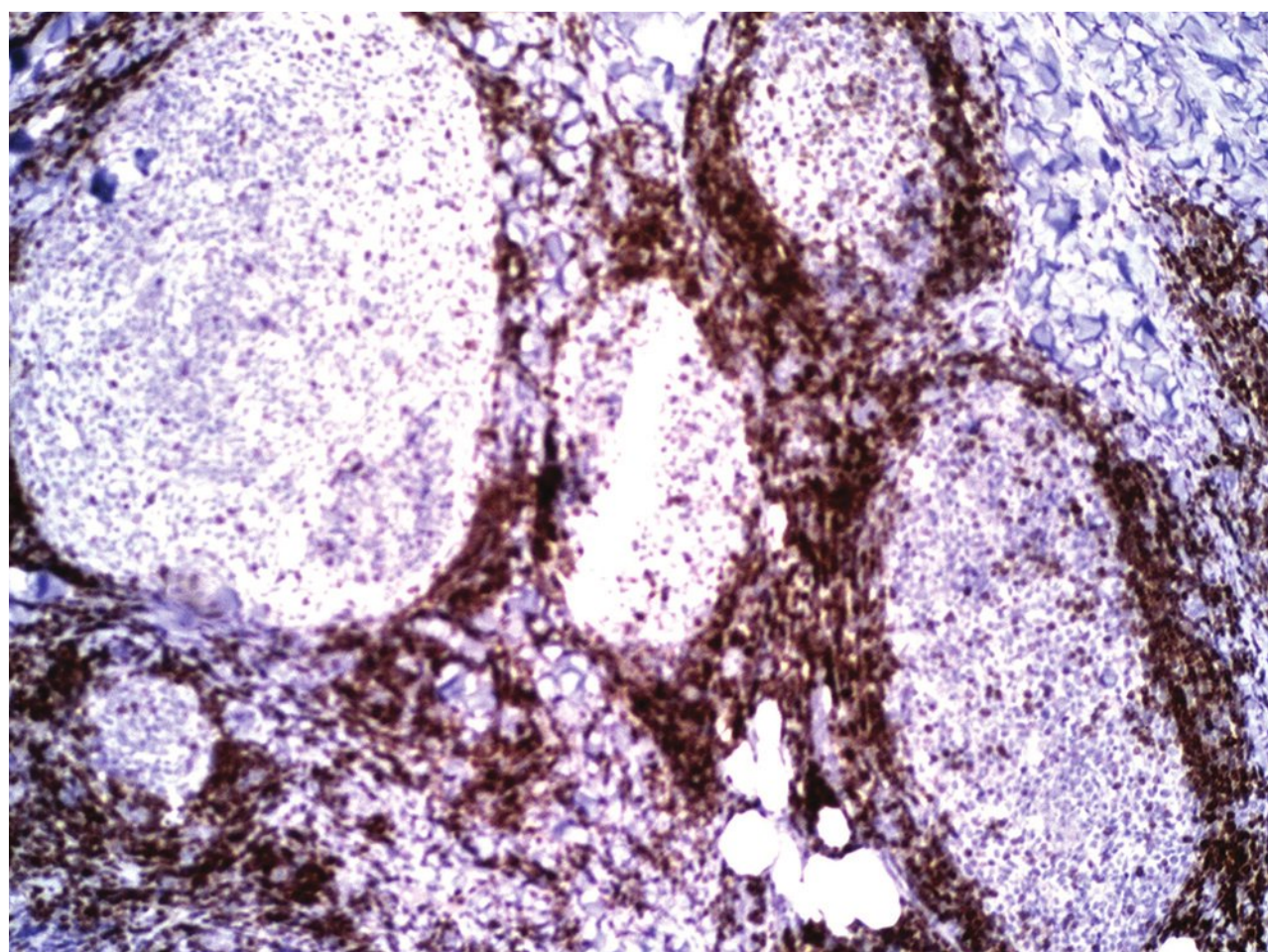


FIGURE 6.30.5 The follicles are negative for BCL-2, which stains the perifollicular T cells. Immunoperoxidase, $\times 10$.

evolve into follicular and diffuse to diffuse pattern during the clinical course. Therefore, clinical presentation, immunophenotyping, or molecular genetic studies are frequently required for a definitive diagnosis. A mantle zone is usually absent in most malignant follicles. In the interfollicular areas, there is often a mixed cellular population consisting of small and large lymphocytes, histiocytes, plasma cells, neutrophils, or eosinophils (5). In the cases with diffuse pattern, lymphoma cells are seen in the interfollicular areas, which can be identified with immunohistochemical staining.

It is important to distinguish secondary follicular lymphoma from PCFCL because there are marked differences in terms of prognosis and treatment between these two entities (6). First of all, a thorough staging is necessary to exclude a nodal follicular lymphoma. Morphologically, a diffuse pattern appears to be more frequently demonstrated in PCFCL than nodal follicular lymphoma (7). Bone marrow involvement is seldom seen in PCFCL, but is common in the nodal tumor.

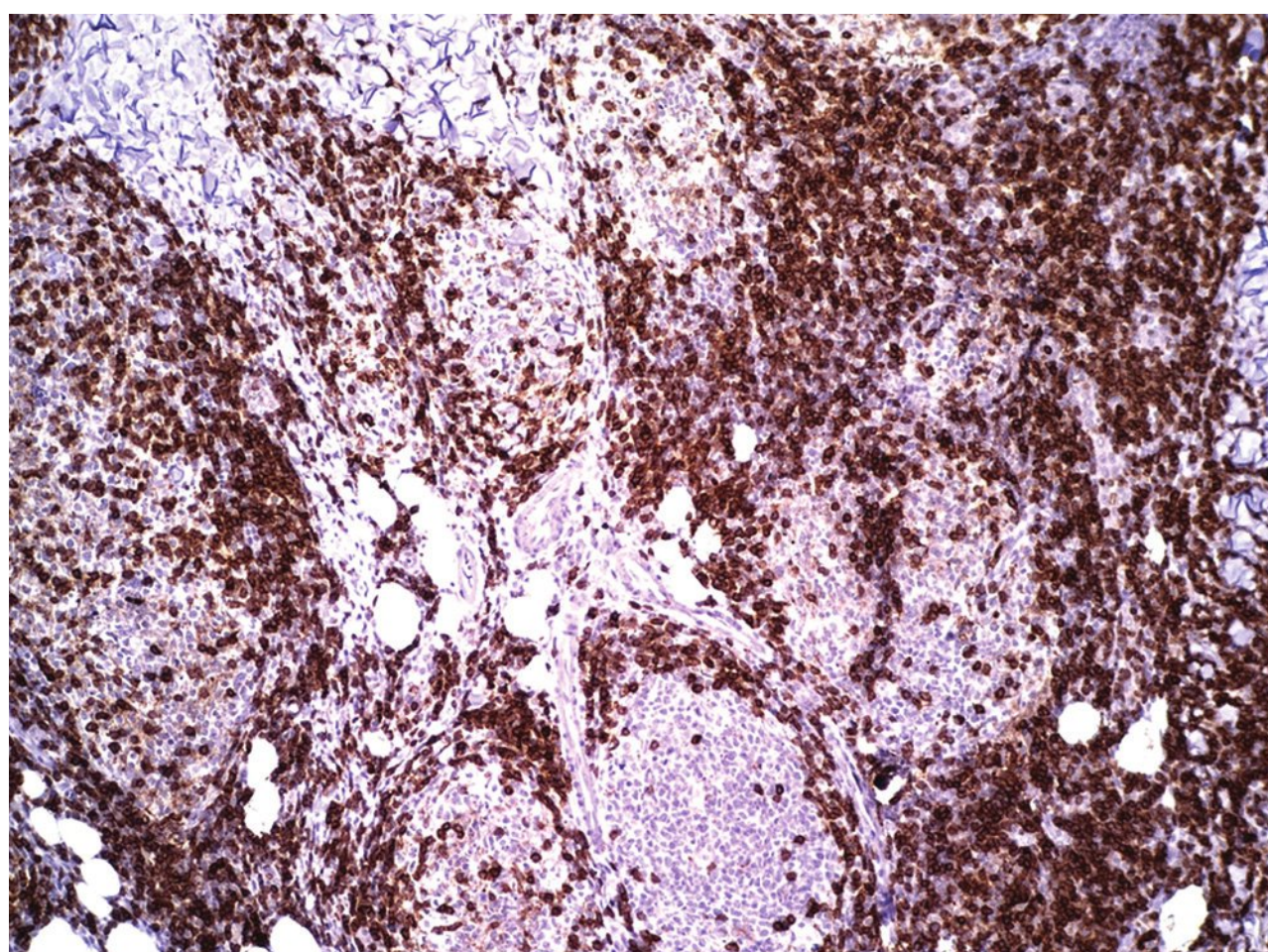


FIGURE 6.30.6 CD3 stains perifollicular T cells and a few intrafollicular T cells. Immunoperoxidase.

Transformation to a high-grade lymphoma is not seen in PCFCL, but it is not uncommon in nodal follicular lymphoma. These two entities also differ in immunophenotypic and molecular genetic characteristics, which will be discussed later.

Another differential diagnosis is primary cutaneous diffuse large B-cell lymphoma (PCDLBCL), leg type, which is defined by a diffuse growth pattern and a monotonous proliferation of centroblasts and immunoblasts, irrespective of site (3,8). In contrast to PCFCL, centrocytes are absent. PCFCL can also be present in the leg, and those cases usually carry a poor prognosis, in spite of its morphologic difference from diffuse large B-cell lymphoma. Cutaneous marginal zone B-cell lymphoma (CMZBCL) is composed of predominantly centrocyte-like B cells without the presence of centroblasts, except in the colonized follicles (3,9). The tumor cells may also assume the morphology of small B lymphocytes or monocytoid B cells. Plasma cell differentiation is frequently present. These entities should be further differentiated with immunophenotyping and/or molecular genetic studies.

Immunophenotype

The most popular monoclonal antibody panel used for differentiation of cutaneous B-cell lymphoma includes CD10, BCL-2, and BCL-6. The 1997 EORTC classification specified negative reactions to CD10 and BCL-2 in the definition of PCFCL (2). Subsequent studies, however, found that CD10 and BCL-2 were present in most cases of PCFCL (5,6,10). The discrepancy is partly explained by the lack of anti-CD10 antibodies applicable to paraffin sections in the early studies, and antibody retrieval methods were not used in the study of BCL-2 (5). The presence of B-cell antigen (CD19, CD20, CD22, and CD79a) and BCL-6 in PCFCL has been universally accepted. The demonstration of follicular dendritic cell network in the tumor nodule by CD21 or CD35 stain is also helpful in the diagnosis. The presence of CD21 staining is controversial in various studies (11,12). One observation is that CD10, BCL-2, and CD21 are more frequently positive in PCFCL cases with a follicular pattern (13).

In secondary cutaneous follicular lymphoma, CD10, BCL-2, and BCL-6 are consistently positive. In CMZBCL, CD10 and BCL-6 are negative, but BCL-2 can be positive in certain cases. In the germinal center–like subtype of diffuse large B-cell lymphoma, the immunophenotype is identical to that of follicular lymphoma. However, its cutaneous counterpart is characteristically CD10 negative (3,7). In addition, MUM1/IRF-4 and FOXP1 are strongly positive in PCDLBCL (3,8). When there is reactive follicular hyperplasia in the skin resembling a lymphoma, it is sometimes referred to as pseudolymphoma. In those cases, CD10 and BCL-6 can be positive, but BCL-2 is always negative. The immunophenotypic differences between these entities are listed in Table 6.30.1 (3,4,8,9).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry may demonstrate a monoclonal B-cell population with coexpression of CD10. This immunophenotype is useful to distinguish a lymphoma from a pseudolymphoma,

TABLE 6.30.1					
Immunophenotypic Differences among Various Cutaneous B-Cell Lymphomas					
	CD10	BCL-2	BCL-6	MUM1/ IRF4	POX-P1
PCFCL	+	+/-	+	-	-
Secondary FL	+	+	+	-	-
PCDLBCL	-	+	+	+	+
CMZBCL	-	+/-	-	-	-
Pseudolymphoma	+	-	+	-	-

CMZBCL, cutaneous marginal zone B-cell lymphoma; PCDLBCL, primary cutaneous diffuse large B-cell lymphoma, leg-type; PCFCL, primary cutaneous follicle center lymphoma; Secondary FL, secondary follicular lymphoma.

but it is not helpful to distinguish different kinds of cutaneous B-cell lymphoma, as discussed above. Therefore, immunohistochemistry is essential to display a complete immunologic panel for differential diagnosis.

Molecular Genetics

The most important molecular genetic study is to demonstrate t(14;18)(q32;q21) by conventional cytogenetic karyotyping or IgH/BCL-2 translocation by fluorescence in situ hybridization (FISH) or PCR. Early studies claim that this karyotype was consistently negative in PCFCL cases (5,14,16), but recent studies with FISH demonstrated many positive cases of PCFCL (6,17). The major explanation is that the primers used in PCR may not cover the specific breakpoint cluster regions in PCFCL, so that the tumor DNA was not amplified (9,17). FISH probes, on the other hand, cover a broad area of breakpoints. Other explanation includes possible BCL-2 mutations or a high load of somatic mutations in those negative cases (17). Case studies with PCR also showed a difference between various grades of PCFCL, the low-grade lymphomas showed a higher positive rate for t(14;18) than that of the high-grade lymphomas (9,18).

The t(14;18) translocation is consistently present in secondary cutaneous follicular lymphoma and occasionally detected in CMZBCL (9). It should not be demonstrated in PCDLBCL and pseudolymphomas (8).

Other rare abnormal karyotypes identified in PCFCL include t(3;14)(q27;q32), t(12;21)(q13;q22), trisomy 17, and deletion of chromosome 14q32.33 (4,17,19). A study of 29 cases of primary cutaneous B-cell lymphomas with FISH for IgH, MYC, BCL-6, and MLT1 loci found that none of the six PCFCL cases and nine CMZBCL cases showed evidence for any translocation affecting these loci (20). In contrast, 11 of 14 PCDLBCL revealed breakpoints in at least one of the loci. However, a recent study with PCR showed that both PCFCL and PCDLBCL cases had aberrant somatic hypermutation of BCL-6, PAX5, RhoH/TTF, and/or MYC (21). A comparative study of 22 PCFCL cases and 13 PCDLBCL cases with comparative genomic hybridization showed that PCFCL cases had fewer imbalances and lacked translocations affecting the IgH locus (22). Gene expression profiling analysis demonstrates that PCFCL

belongs to the germinal center-like B-cell subtype (4). On the other hand, PCDLBCL shows an activated B-cell-like pattern (8).

The salient features for laboratory diagnosis of PCFCL are summarized in Table 6.30.2.

Clinical Manifestations

The importance of separating PCFCL from other cutaneous B-cell lymphomas is due to its indolent clinical course with local symptoms and favorable prognosis, thus requiring more conservative treatment than other entities. Most cases only require restricted field radiotherapy. PCFCL may have relapses but local treatment usually achieves long-term remission. PCFCL seldom involves other organs, such as liver, spleen, and bone marrow. Secondary lymph node involvement has been reported in only rare cases (23). Transformation to high-grade lymphomas has not been reported in PCFCL cases.

PCFCL is usually seen in the elderly with a mean age varying from 51 to 63 in different reports. The lesion is often present in the head and neck region, followed by the trunk. It is usually in the form of erythematous to violaceous plaques, nodules, or tumors of variable size. The

TABLE 6.30.2	
Salient Features for Laboratory Diagnosis of PCFCL	
1. Follicular, follicular and diffuse, or diffuse pattern, consisting of centrocytes and centroblasts in cutaneous dermis.	
2. Tumor cells are positive for B-cell antigens (CD19, CD20, CD22, CD79a), CD10, and BCL-6, but BCL-2 can be positive or negative.	
3. Follicles contains a follicular dendritic meshwork, demonstrated by CD21 or CD35	
4. IgH/BCL-2 or t(14;18)(q32;q21) can be demonstrated by FISH or PCR in a subset of PCFCL	
5. Gene expression profiling identifies a germinal center-like B-cell pattern	

5-year survival rate is over 95% (4). However, if the lesion is located in the leg, the prognosis is often worse than those in other sites (24).

The current case showed only a single skin lesion with one episode of relapse on the forehead but no involvement of lymph nodes and other organs. PET/CT scan showed no extracutaneous lesions. He received restricted field radiotherapy and maintained a long-term remission. The tumor cells were positive for CD20, CD10, and BCL-6, but were negative for BCL-2 and T-cell markers. The t(14;18) translocation by PCR was negative. The immunophenotype and karyotype helped exclude other cutaneous B-cell lymphomas. Therefore, this is a typical case of PCFCL.

REFERENCES

1. Ziemer M, Bauer H, Fluhr JW, et al. Primary cutaneous follicle center lymphoma—"Crosti lymphoma". *Am J Clin Dermatol*. 2008;9:133-136.
2. Willemze R, Kerl H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the cutaneous lymphoma study group of the European Organization for Research and Treatment of Cancer. *Blood*. 1997;90:354-371.
3. Burg G, Kempf W, Cozzio A, et al. WHO/EORTC classification of cutaneous lymphomas 2005: histological and molecular aspects. *J Cutan Pathol*. 2005;32:647-674.
4. Willemze R, Swerdlow SH, Harris NL, et al. Primary cutaneous follicle centre lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:227-228.
5. Goodlad JR, Krajewski AS, Batstone PJ, et al. Primary cutaneous follicular lymphoma: a Clinicopathologic and molecular study of 16 cases in support of a distinct entity. *Am J Surg Pathol*. 2002;26:733-741.
6. Kim BK, Surti U, Pandya A, et al. Clinicopathologic, immunophenotypic, and molecular cytogenetic fluorescence in situ hybridization analysis of primary and secondary cutaneous follicular lymphomas. *Am J Surg Pathol*. 2005;29:69-82.
7. Anghel G, Pulsoni A, de rosa L. Primary cutaneous follicle center cell lymphoma and limited stage follicular non-Hodgkin's lymphoma: A comparison of clinical and biological features. *Leuk Lymphoma*. 2002;43:2109-2115.
8. Meijer CJLM, Vergier B, Duncan LM, et al. Primary cutaneous DLBCL, leg type. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:242.
9. Isaacson PG, Chott A, Nakamura S, et al. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:213-217.
10. Lawnicki LC, Weisenburger DD, Aoun P, et al. The t(14;18) and bcl-2 expression are present in a subset of primary cutaneous follicular lymphoma. Association with lower grade. *Am J Clin Pathol*. 2002;118:765-772.
11. Cerroni L, Kerl H. Primary cutaneous follicle center cell lymphoma. *Leuk Lymphoma*. 2001;42:891-900.
12. Hoefnagel JJ, Vermeer MH, Jansen PM, et al. Bcl-2, Bcl-6 and CD10 expression in cutaneous B-cell lymphoma: further support for a follicle centre cell origin and differential diagnostic significance. *Br J Dermatol*. 2003;149:1183-1191.
13. Kerl H, Cerroni L, Fink-Puches R, et al. Primary cutaneous B-cell lymphomas [in German]. *J Dtsch Dermatol Ges*. 2004;2:605-620.
14. Cerroni L, Volkenandt M, Rieger E, et al. Bcl-2 expression and correlation with the interchromosomal 14;18 translocation in cutaneous lymphomas and pseudolymphomas. *J Invest Dermatol*. 1994;102:231-235.
15. Child FJ, Russell-Jones R, Woolford AJ, et al. Absence of the t(14;18) chromosomal translocation in primary cutaneous B-cell lymphoma. *Br J Dermatol*. 2001;144:735-744.
16. Franco R, Fernandez-Vazquez A, Rodriguez-Peralto JL, et al. Cutaneous follicular B-cell lymphoma: description of a series of 18 cases. *Am J Surg Pathol*. 2001;25:875-883.
17. Streubel B, Scheuchner B, Valencak J, et al. Molecular cytogenetic evidence of t(14;18)(IGH;BCL2) in a substantial proportion of primary cutaneous follicle center lymphomas. *Am J Surg Pathol*. 2006;30:529-536.
18. Estalilla OC, Medeiros LJ, Manning JT Jr, et al. 5',3' Exonuclease-based real-time PCR assays for detecting the t(14;18)(q32;21): a survey of 162 malignant lymphomas and reactive specimens. *Mod Pathol*. 2000;2:86-102.
19. Jelic TM, Berry PK, Jubelirer SJ, et al. Primary cutaneous follicle center lymphoma of the arm with a novel chromosomal translocation t(12;21)(q13;q22): a case report. *Am J Hematol*. 2006;81:448-453.
20. Hellermann C, Kaune KM, Gesk S, et al. Molecular cytogenetic analysis of chromosomal breakpoints in the IGH, MYC, BCL6, and MALT1 gene loci in primary cutaneous B-cell lymphomas. *J Invest Dermatol*. 2004;123:213-219.
21. Dijkman R, Tensen CP, Buettner M, et al. Primary cutaneous follicle center lymphoma and primary cutaneous large B-cell lymphoma, leg type, are both targeted by aberrant somatic hypermutation but demonstrate differential expression of AID. *Blood*. 2006;107:4926-4929.
22. Hellermann C, Kaune KM, Siebert R, et al. Chromosomal aberration patterns differ in subtypes of primary cutaneous B cell lymphomas. *J Invest Dermatol*. 2004;122:1495-1502.
23. Serooskerken AMVTV, Mosterd K, Veraart JC, et al. Coincidence of cutaneous follicle center lymphoma and diffuse large B-cell lymphoma. *Int J Dermatol*. 2008;47(suppl 1):21-24.
24. Kodama K, Massone C, Chott A, et al. Primary cutaneous large B-cell lymphomas: Clinicopathologic features, classification, and prognostic factors in a large series of patients. *Blood*. 2005;106:2491-2497.

CASE 31

Mantle Cell Lymphoma

CASE HISTORY

A 64-year-old man was diagnosed with chronic lymphocytic leukemia (CLL) and was treated with chemotherapy for 1 year. Three years later, the patient developed splenomegaly and splenectomy with a hilar lymph node biopsy was performed. After 2 years, he was referred to another hospital. Upon reviewing the splenectomy specimen and lymph node biopsy, with further flow cytometric analysis of the peripheral blood, the pathologist in the second hospital considered this case to be mantle cell lymphoma (MCL). This new diagnosis was confirmed by a fluorescence in situ hybridization (FISH) study, which demonstrated IgH/BCL-1 translocation.

Subsequently, the patient had a relatively stable clinical course. However, 9 years after the initial diagnosis, his leukocyte count was elevated to 120,000/mL with the presence of blastoid cells. A bone marrow biopsy confirmed blastoid transformation. The patient was started with a course of oral chlorambucil to no avail. A computed tomography scan revealed abdominal lymphadenopathy. He began to experience diplopia, and examination of cerebrospinal fluid demonstrated lymphoma cells. Chest x-ray also revealed pulmonary infiltration with bilateral pleural effusion. Bronchoalveolar lavage showed lymphoma cells with an immunophenotype of MCL by flow cytometry. Despite aggressive chemotherapy, the patient's condition deteriorated rapidly and he died 9 months after the finding of blastoid cells in the peripheral blood. An autopsy was performed and disclosed extensive tumor dissemination, involving multiple abdominal lymph nodes, the lungs, kidneys, liver, small intestine, colon, testes, prostate gland, adrenal glands, and spinal cord.

FLOW CYTOMETRIC FINDINGS

Peripheral blood: CD5 90%, CD19 96%, CD19/CD5 92%, CD20 99%, CD23 4%, CD10 2%, FMC-7 98%, CD19/k 98%, CD19/l 2% (Fig. 6.31.1).

Bronchoalveolar lavage: CD5 96%, CD19 91%, CD19/CD5 91%, CD20 94%, CD23 3%, CD10 4%, FMC-7 94%, CD19/k 88%, CD19/l 2%.

CYTOGENETIC STUDIES

Karyotype of the bone marrow showed 46,XY,t(11;14)(q13;q32)[2]/46,XY[18]. FISH of the bone marrow revealed 56.1% of cells with a cyclin D1-IgH gene fusion with apparent clonal evolution. It was reported as: nuc ish 11q13 (CCND1×3),14q32(IgH×3) (CCND1 con IgH×1)[17]/11q13 (CCND1×2),14q32(IgH×2)[36].

DISCUSSION

MCL was initially designated by Berard and Dorfman as lymphocytic lymphoma of intermediate differentiation for a group of non-Hodgkin lymphomas that could not be classified into either well-differentiated or poorly differentiated lymphocytic lymphomas according to Rappaport's classification (1). Because about 50% of lymphocytic lymphoma of intermediate differentiation cases showed membrane-associated alkaline phosphatase activity, which is normally found on the membrane of follicular cuff lymphocytes, the alternative term mantle zone lymphoma was proposed for this tumor (1). It was later found that mantle zone lymphoma was identical to centrocytic lymphomas in the Kiel classification (2). In 1982, the International Lymphoma Study Group renamed this tumor MCL lymphoma (3), a term that has since been accepted by the Revised European American Classification of Lymphoid Neoplasms (4) and the World Health Organization (WHO) Classification of Tumours of Hematopoietic and Lymphoid Tissues (5,6).

Morphology

There are four histologic patterns of MCL: diffuse, mantle zone, nodular and rarely, follicular (1–3,5). The diffuse type is most frequently encountered and is usually difficult to diagnose without immunophenotyping (Fig. 6.31.2). The mantle zone type shows a residual or a naked germinal center surrounded by an expanded mantle zone (Fig. 6.31.3), and the nodular type may represent colonization of the germinal center (7) or arise from the primary lymphoid follicle (Fig. 6.31.4) (1). Some authors suggest that mantle zone, nodular and diffuse patterns may represent three developmental stages of the disease, as they can be demonstrated in the same patients in consecutive biopsies (8). In occasional cases, only a thin rim of tumor cells is demonstrated in the mantle zone by cyclin D1 stain. These cases show no interfollicular involvement and no extranodal dissemination and are designated “in situ” MCL (6,8,9).

According to the WHO classification, there are three morphologic variants: small cell, marginal zone-like B-cell, and aggressive type, in addition to the classical centrocyte-like morphology (5,6). The small-cell variant shows slight-to-moderate nuclear irregularity or cleft, with clumped chromatin pattern, no nucleoli, and scant cytoplasm. The marginal zone-like B-cell has abundant pale cytoplasm resembling the tumor cells of marginal zone

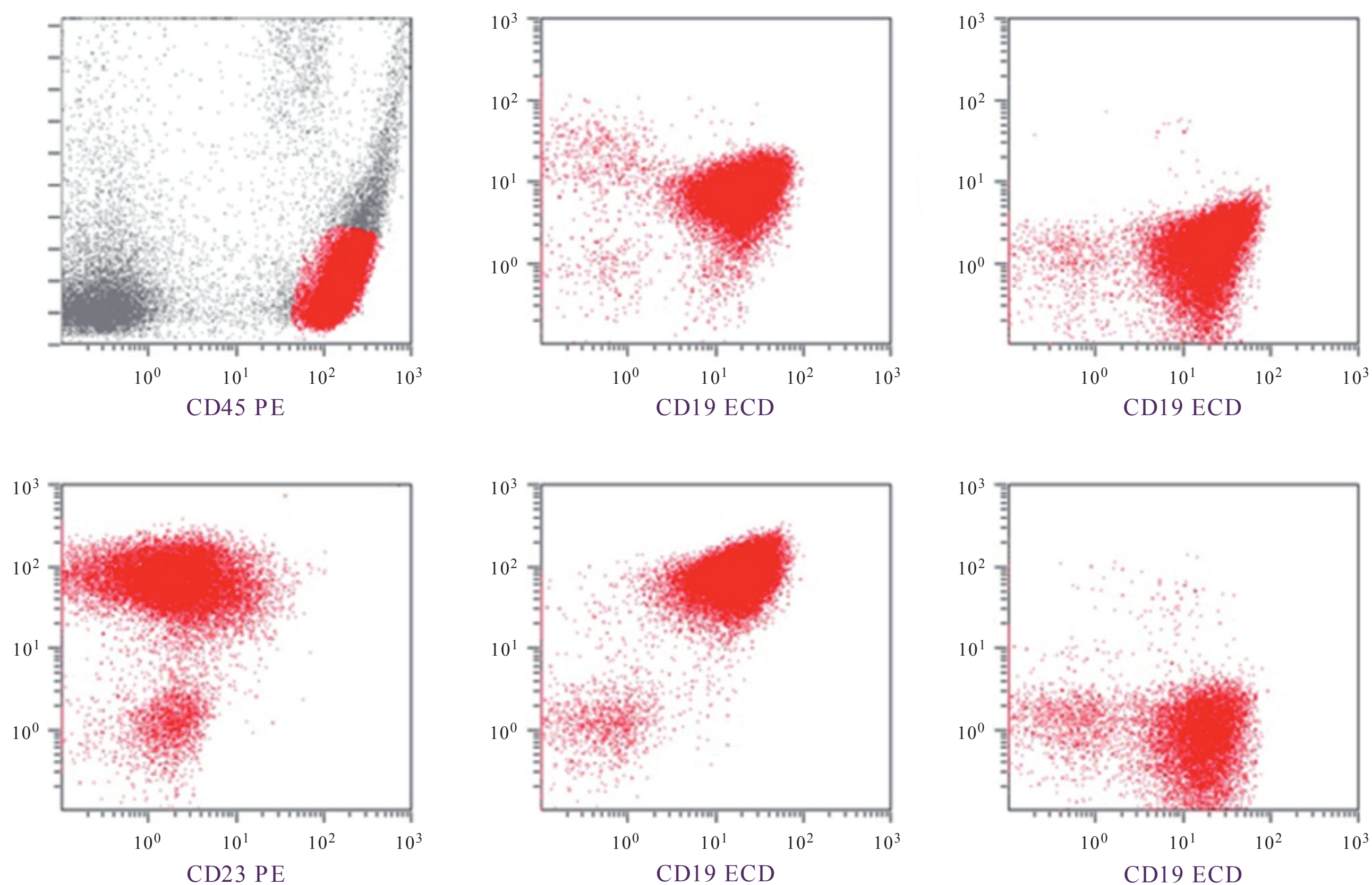


FIGURE 6.31.1 Flow cytometric histograms show dual CD19/CD5 staining and positive FMC-7 in a monoclonal κ population. CD23 is partial positive and CD10 is negative. SS, side scatter; FITC, fluorescein isothiocyanate; ECD, phycoerythrin-Texas Red; PE, phycoerythrin.

B-cell lymphoma. The aggressive variant is divided into blastoid and pleomorphic subtypes. Tumor cells in the blastoid subtype resemble lymphoblasts with dispersed chromatin and a high mitotic rate (Fig. 6.31.5). Tumor cells in the pleomorphic subtype are heterogeneous with large

cleaved to oval nuclei and pale cytoplasm. Nucleoli may be prominent in this subtype (5).

The European MCL Network studied 304 MCL patients and divided MCL into the following subtypes: classical (87.5%), small cell (3.6%), pleomorphic (95.9%), and blastic

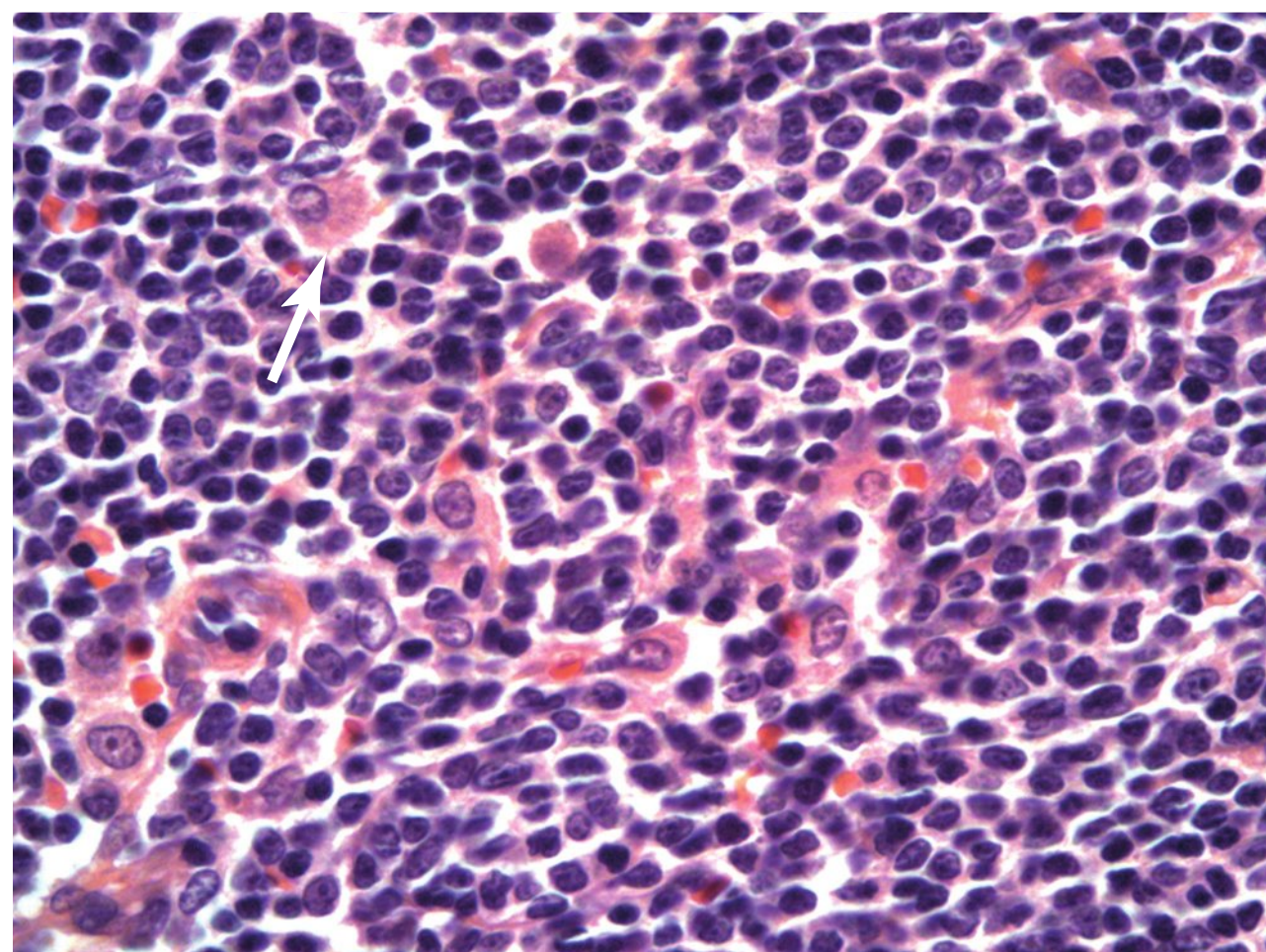


FIGURE 6.31.2 Diffuse type of MCL shows diffuse infiltration of small lymphoid cells with slightly irregular nuclear configuration. Multiple pink histiocytes (white arrow) are present. Hematoxylin and eosin, 60× magnification.

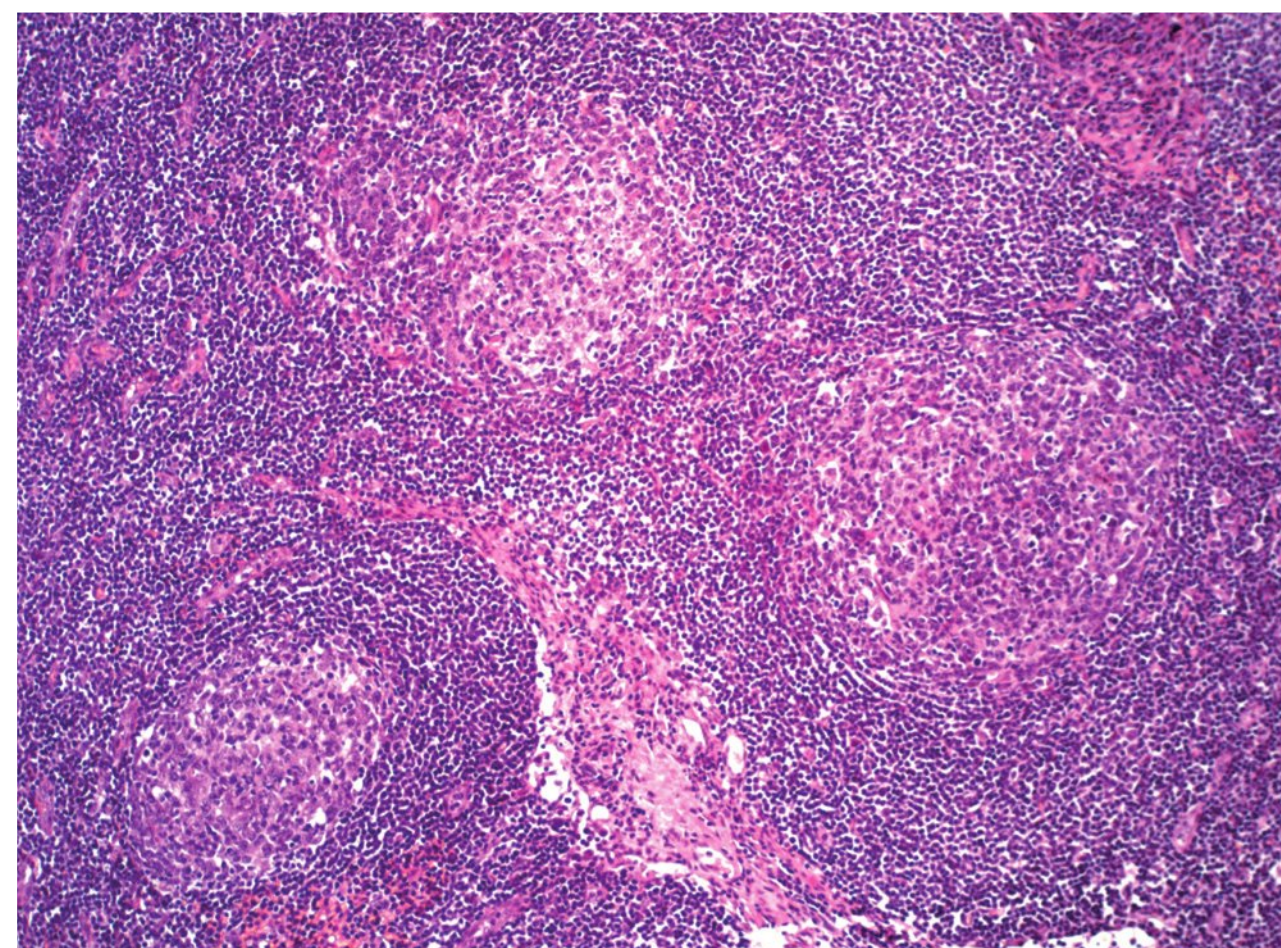


FIGURE 6.31.3 Mantle zone type of MCL reveals residual germinal centers with greatly expanded mantle zones, which coalesce with each other. Hematoxylin and eosin, 10× magnification.

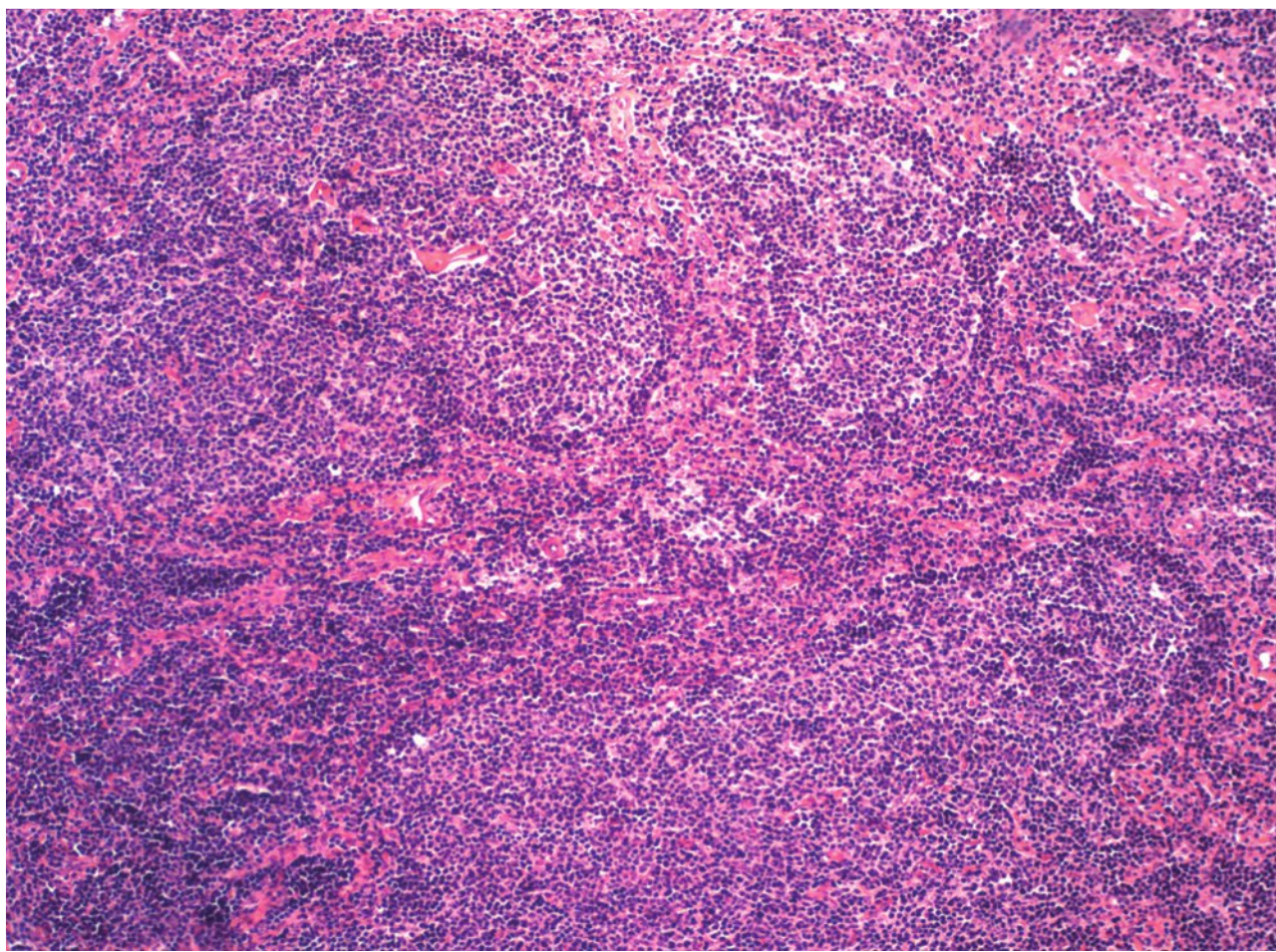


FIGURE 6.31.4 Nodular type of MCL shows multiple nodules with irregular margins. Hematoxylin and eosin, 10× magnification.

(2.6%) (10). The classical subtype is equivalent to the centrocytic subtype in WHO classification. No marginal zone-like subtype was included in this study.

The characteristic morphologic features of MCL are summarized in Table 6.31.1.

In about two thirds of the cases, histiocytes with granular eosinophilic cytoplasm (pink histiocytes) are intermingled with the tumor cells, imparting a “starry sky” pattern (11). Unlike the tingible-body macrophages seen in Burkitt lymphoma and lymphoblastic lymphoma, these histiocytes do not contain nuclear debris.

One study showed that, in the bone marrow, 82% had a nodular pattern, 50% interstitial, 45% paratrabeular, and 32% diffuse (12). Another study revealed a similar order of frequency, with 68% showing nodular or interstitial pattern, 46% paratrabeular, and 23% diffuse (13). In

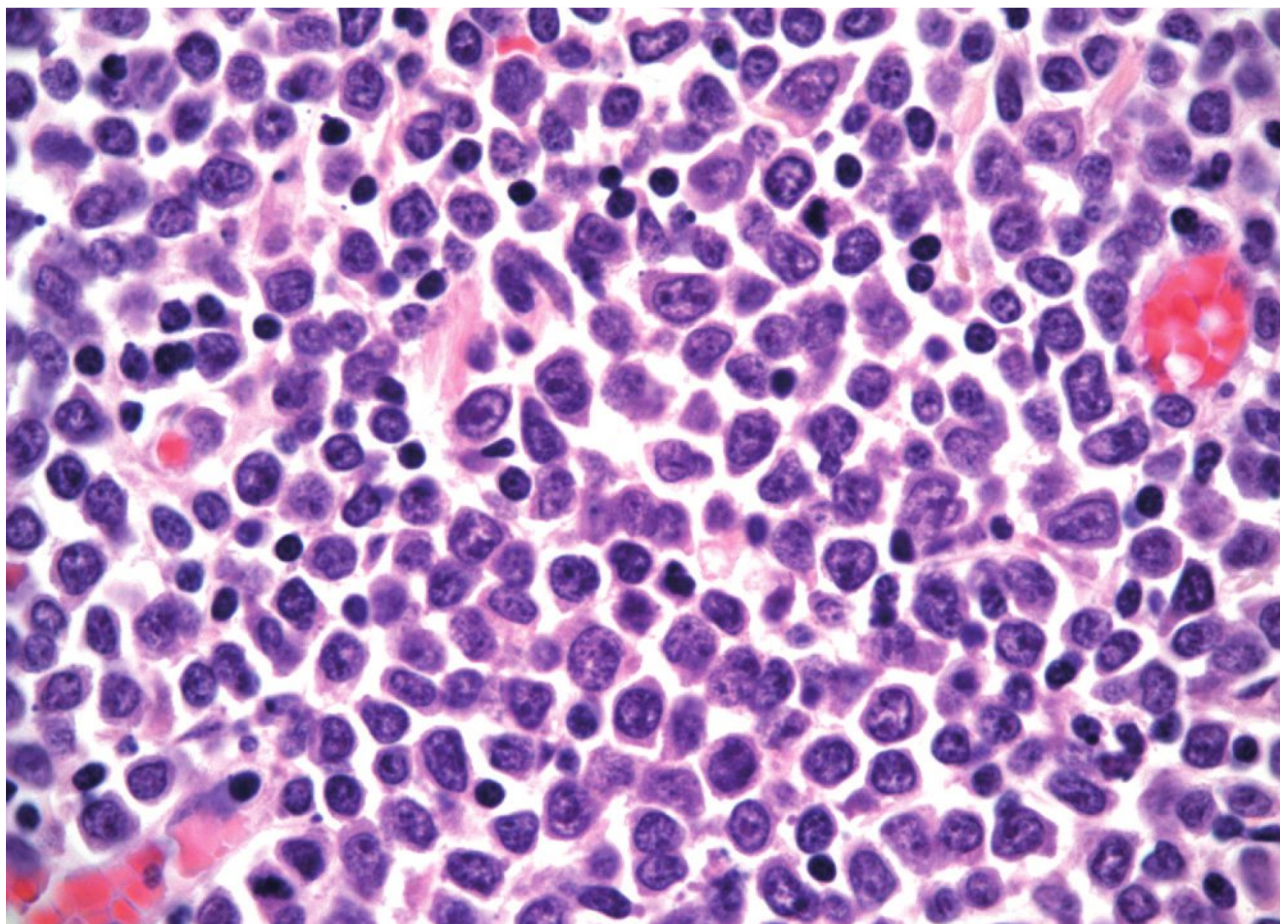


FIGURE 6.31.5 Blastoid variant of MCL reveals medium-sized lymphoid cells with large irregular nuclei and a dispersed chromatin pattern. Nucleoli are seen in a few tumor cells. Please compare with Figure 6.31.2. Hematoxylin and eosin, 60× magnification.

TABLE 6.31.1

Characteristic Morphologic Features of MCL

Histologic pattern	Diffuse, mantle zone, and nodular
Cytology	Centrocytes, small cell, marginal zone B-cell, blastoid, and pleomorphic
Specific features	Naked germinal center surrounded by an expanded mantle zone in mantle zone subtype; scattered “pink histiocytes” in diffuse subtype

the spleen, the white pulp is markedly expanded by the tumor cells, but reactive follicles with prominent germinal centers may be also coexistent (14). Liver involvement is mainly confined to the portal areas (Fig. 6.31.6).

Lymphomatous polyposis of the gastrointestinal tract is most frequently due to MCL (Figs. 6.31.7 and 6.31.8) (15,16). When MCL is present in the gastrointestinal tract, polyposis is the most common presentation.

MCL may mimic several non-Hodgkin lymphomas (14). When the diffuse type of MCL shows minimal nuclear irregularity, it should be distinguished from small lymphocytic lymphoma (SLL). The latter is characterized by the presence of proliferation centers, which should be distinguished from the residual germinal centers in the mantle zone type of MCL (3). The residual germinal centers contain a mixture of small and large centrocytes and centroblasts (Fig. 6.31.9), but the proliferation centers contain prolymphocytes and paraimmunoblasts. When MCL shows a nodular pattern, it should be distinguished from follicular lymphoma (FL), and the diffuse type of MCL

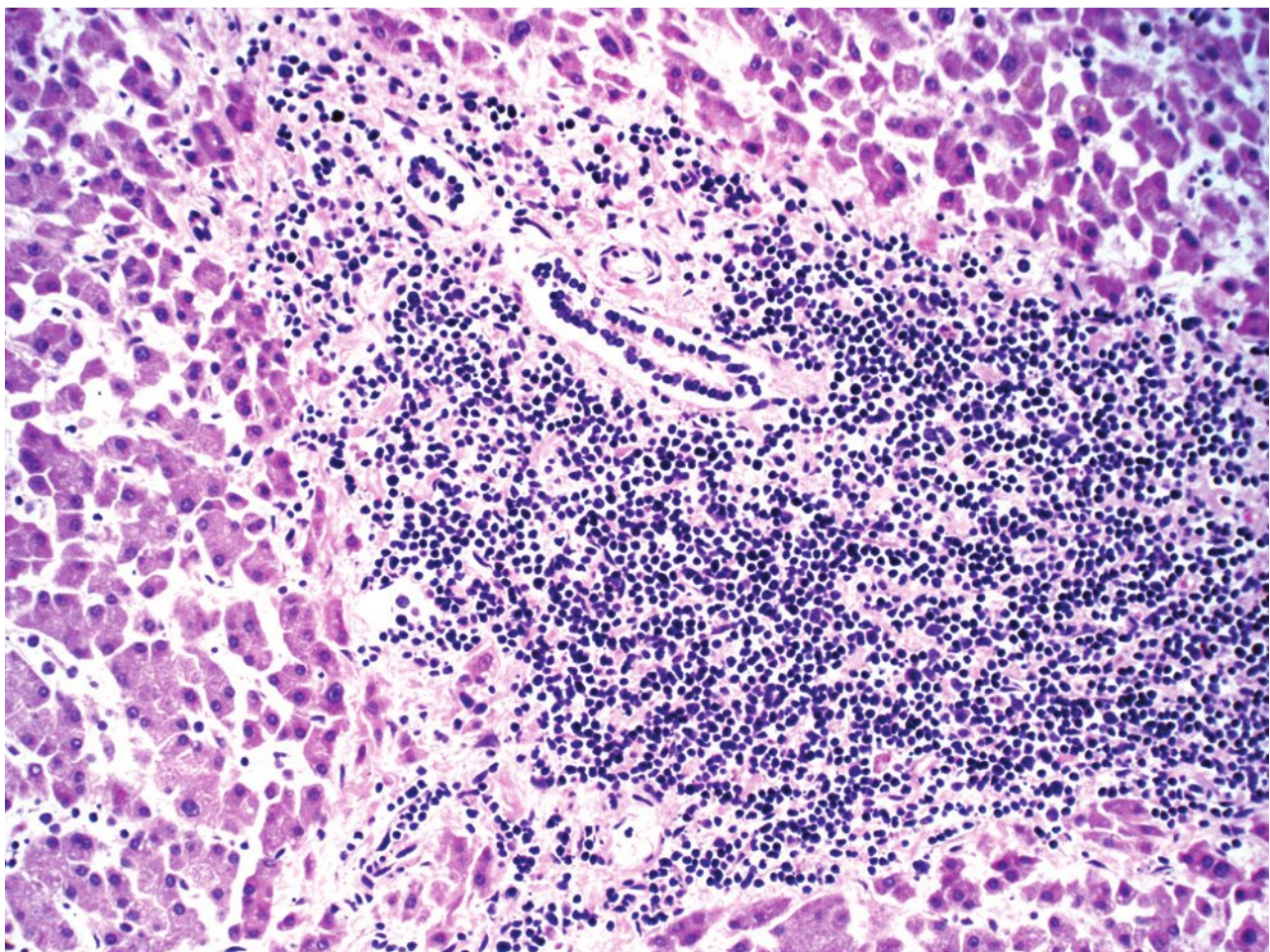


FIGURE 6.31.6 Liver biopsy shows lymphomatous infiltration in the portal area with expansion to the adjacent liver lobules.

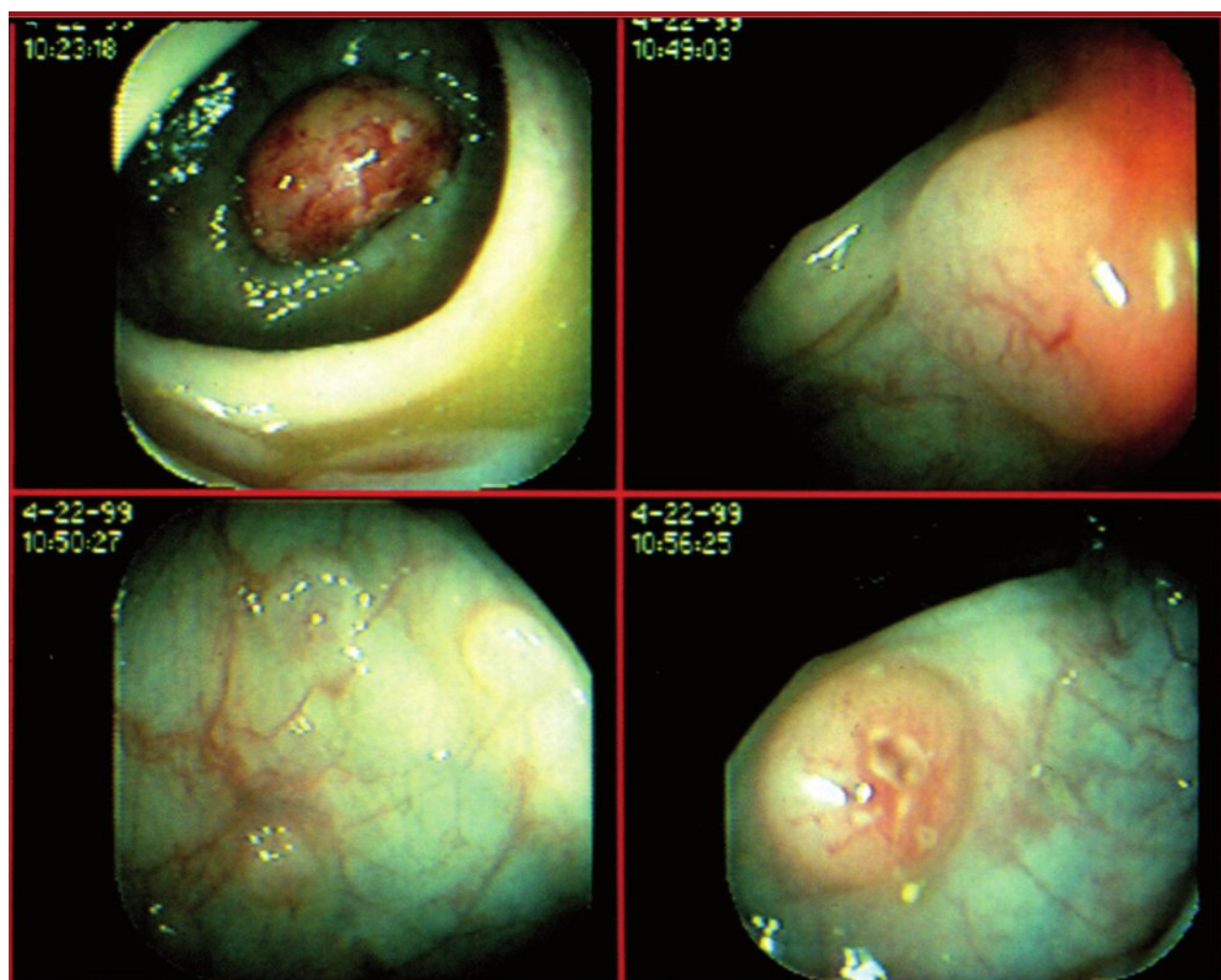


FIGURE 6.31.7 Colonoscopy demonstrates a few polyps with ulcerative surface. (Courtesy of Dr. Stacy Weiland, VA Medical Center, Denver, CO.)

should be distinguished from the diffuse type of FL. Large transformed cells with vesicular nuclei and prominent nucleoli and small cells with markedly cleaved nuclei are usually present in both types of FL, but are not seen in MCL (14,17).

The aggressive variant of MCL can be divided into the blastoid and pleomorphic subtypes, which may mimic lymphoblastic lymphoma or large-cell lymphoma, respectively (18,19). Their distinction is mainly based on immunophenotyping. Finally, when a leukemic phase of MCL appears (Fig. 6.31.10), the differential diagnoses include CLL, prolymphocytic leukemia, acute leukemia, leukemic phase of FL, and leukemic phase of diffuse large B-cell lymphoma (20). The differences between MCL, FL, and SLL/CLL are summarized in Table 6.31.2.

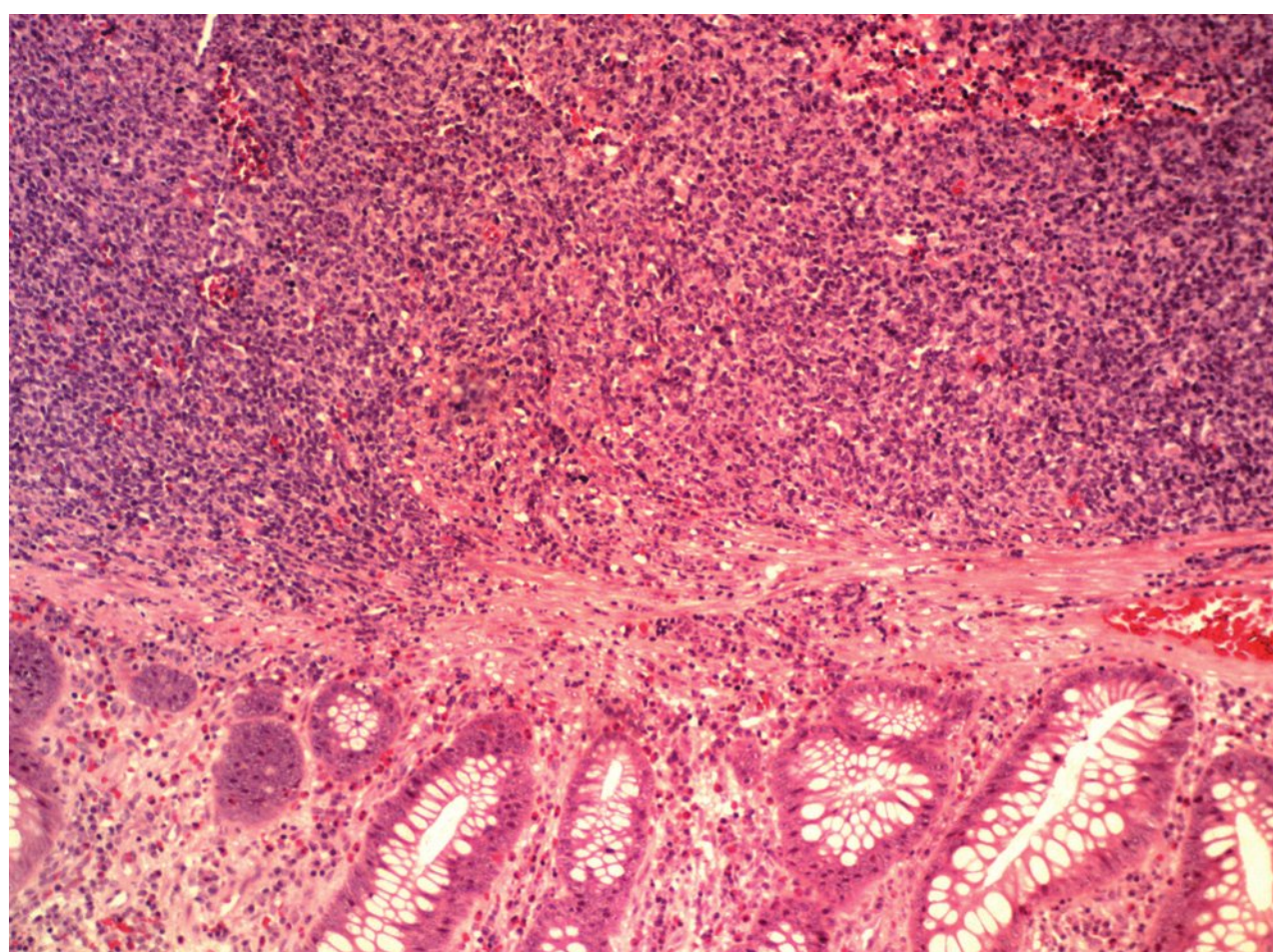


FIGURE 6.31.8 Colonic polyp shows extensive lymphoma cell infiltration in the submucosa. The lamina propria is not involved. Hematoxylin and eosin, 10× magnification.

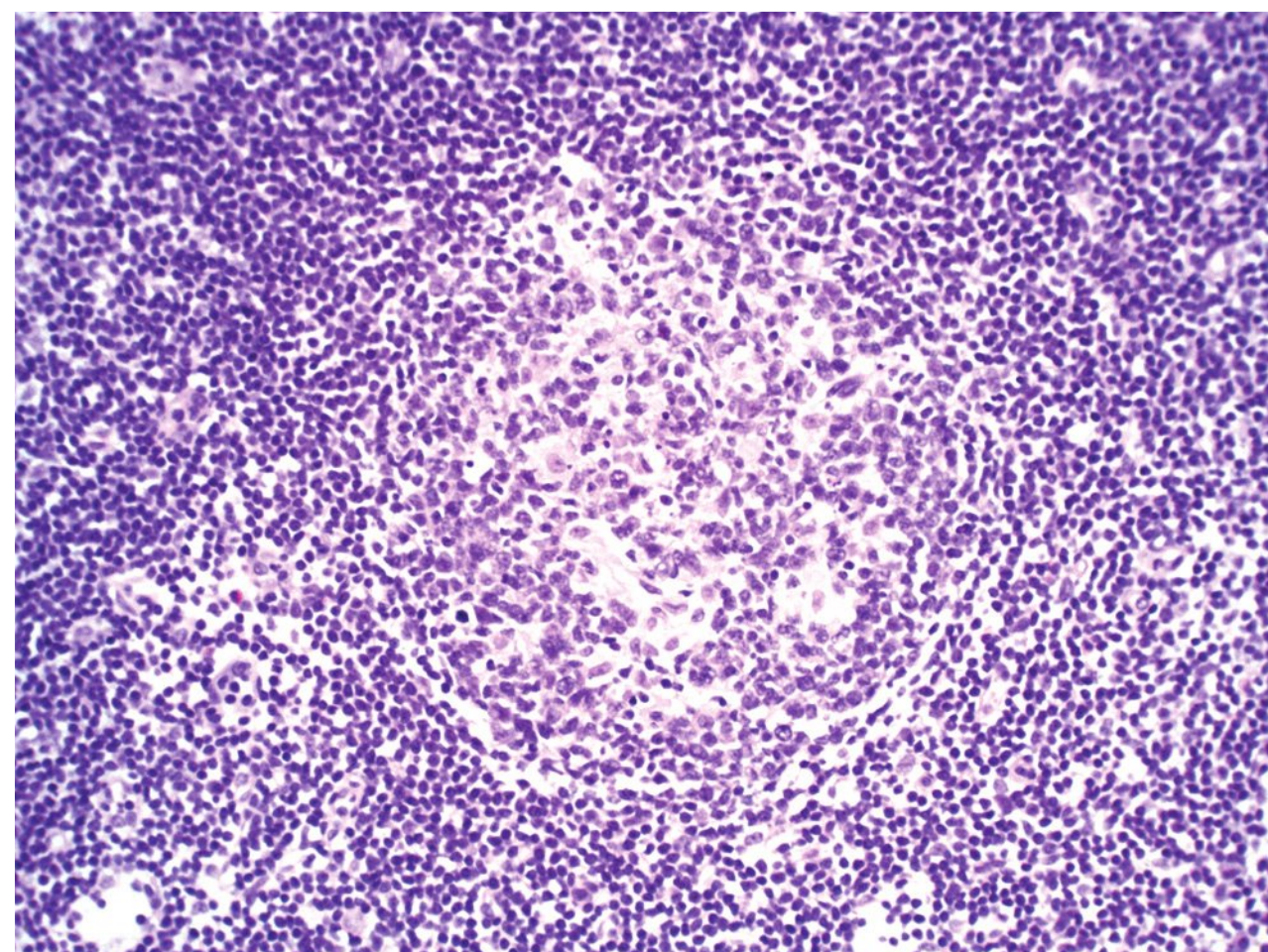


FIGURE 6.31.9 Lymph node biopsy shows a residual germinal center with well-demarcated margin and mixtures of centrocytes and centroblasts.

Immunophenotype

Immunophenotyping is most helpful in distinguishing MCL from other lymphomas. However, the immunophenotype of SLL/CLL is similar to that of MCL. Both groups have coexpression of pan-B-cell antigens (CD19 or CD20) with a T-cell antigen, CD5 (Fig. 6.31.11) (11,14). The major difference is the reaction to CD23: It is negative for MCL but positive for SLL/CLL. However, CD23-positive cases (21–23) and CD5-negative cases (24) of MCL have been reported. In addition, CD22 is positive in MCL cases but is negative in most cases of SLL/CLL. MCL also has brighter staining than SLL/CLL for surface Ig and CD20. FMC-7 is frequently demonstrated in MCL, but not in SLL/CLL cases unless prolymphocytic transformation occurs. The follicular dendritic cells, as demonstrated by CD21, are present in MCL and not in SLL/CLL (1). In contrast to most lymphomas and leukemias, two thirds of MCL cases express surface λ rather than κ light chain.

The distinction of MCL from FL is mainly based on the reactions to CD5 and CD10: MCL is positive for CD5 but negative for CD10; the opposite is true for FL. Although some studies showed that blastoid MCL cases were less likely to express CD5 and may express CD10, most investigators found that the blastoid subtype had a phenotype similar to that of other subtypes of MCL (18–20). In immunohistochemical studies, there are additional markers that may help to distinguish MCL from FL (14,25,26). MCL is positive for IgD, CD43, CD74 (LN2), CD62L (Leu-8), and CD79b but negative for CD45RA and CDw75 (LN1). The phenotype of FL in these studies is IgD–, CD43–, CD74+, CD62L+, CD79b–, CD45RA+, CDw75+. The standard immunophenotype for FL is CD10+, bcl-2+, bcl-6+.

The presence of surface Ig and absence of terminal deoxynucleotidyl transferase and CD10 in blastoid MCL are helpful in distinguishing it from lymphoblastic lymphoma. In fact, most cases of lymphoblastic lymphoma are of T-cell lineage, so the presence of several T-cell markers can readily distinguish lymphoblastic lymphoma from



TABLE 6.31.2

Differentiation between MCL, FL, and SLL/CLL

	MCL	FL	SLL/CLL
Cell size	Intermediate	Small	Small to large
Surface Ig	IgM-l (2/3)	IgM-k	IgM-k
Fluorescence intensity	Moderate	Bright	Dim
CD5	+	—	+
CD10	—	+	—
CD19	+	+	+
CD20	+	+	+
CD22	+	+	±
HLA-DR	+	+	+
CD23	—	—	+
CD43	+	—	+
CD45RA	—	+	—
CD74	+	+	+
CDw75	—	+	—
CD79b	+	—	—
CD62L	+	±	+
IgD	+	—	+
Cyclin D1	+	—	—
Alkaline phosphatase	+	—	—
Cytogenetics	t(11;14)	t(14;18)	+12
Proto-oncogene	bcl-1	bcl-2	None

CLL, chronic lymphocytic leukemia; FL, follicular lymphoma; Ig, immunoglobulin; MCL, mantle cell lymphoma; SLL, small lymphocytic lymphoma

MCL, which is of B-cell lineage. Diffuse large B-cell lymphoma may mimic the pleomorphic subtype of MCL but is usually CD5 negative (20), although CD5-positive diffuse large B-cell lymphoma has been reported (27,28).

Cyclin D1 protein as demonstrated by immunohistochemical stains has been considered the most reliable marker for MCL (Figs. 6.31.12 and 6.31.13). The positive rates varied from 72% to 100% in MCL cases, but it can be also positive in a few cases of prolymphocytic leukemia, splenic lymphoma with villous lymphocytes, and occasional SLL/CLL, hairy cell leukemia, and plasmacytoma/myeloma (27,29). Cyclin D1 has not been found in FL, nodal marginal zone B-cell lymphoma, lymphoplasmacytic lymphoma, Sézary syndrome, reactive lymphoid hyperplasia, or high-grade lymphomas (29,30). Cases with positive cyclin D1 staining generally have worse prognosis than those with negative staining (31,32).

Cyclin D1 also can be detected by flow cytometry, but it has been used only on a research basis (33). Similar to immunohistochemical staining, cyclin D1 has been demonstrated in subsets of CLL, prolymphocytic leukemia, hairy cell leukemia, and multiple myeloma by flow cytometry.

Two prognostic predictors, Ki-67 and survivin, can be demonstrated by immunohistochemistry. In patients with Ki-67 expression in >50% of tumor cells, the median survival is 9 months. In contrast, the median survival is 62 months for patients with expression in <50% (34). Survivin is a member of the inhibitor of apoptosis protein family that is expressed in G2/M phase. Cases with nuclear survivin expression in >20% of tumor cells have a survival time of 8 months; it is 60 months for those with expression in <20% (34).

MCL cells may also express surface CD40. The importance of detecting CD40 is that CD40-ligating agents, such as CD40 ligand (CD40L) and CD40 monoclonal antibody, can be used for immunotherapy of MCL, and the increase of soluble CD40 in a patient's blood is associated with a poor prognosis (35).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is able to distinguish MCL from other lymphomas in most cases, but immunohistochemistry is more reliable because there are more markers available for this

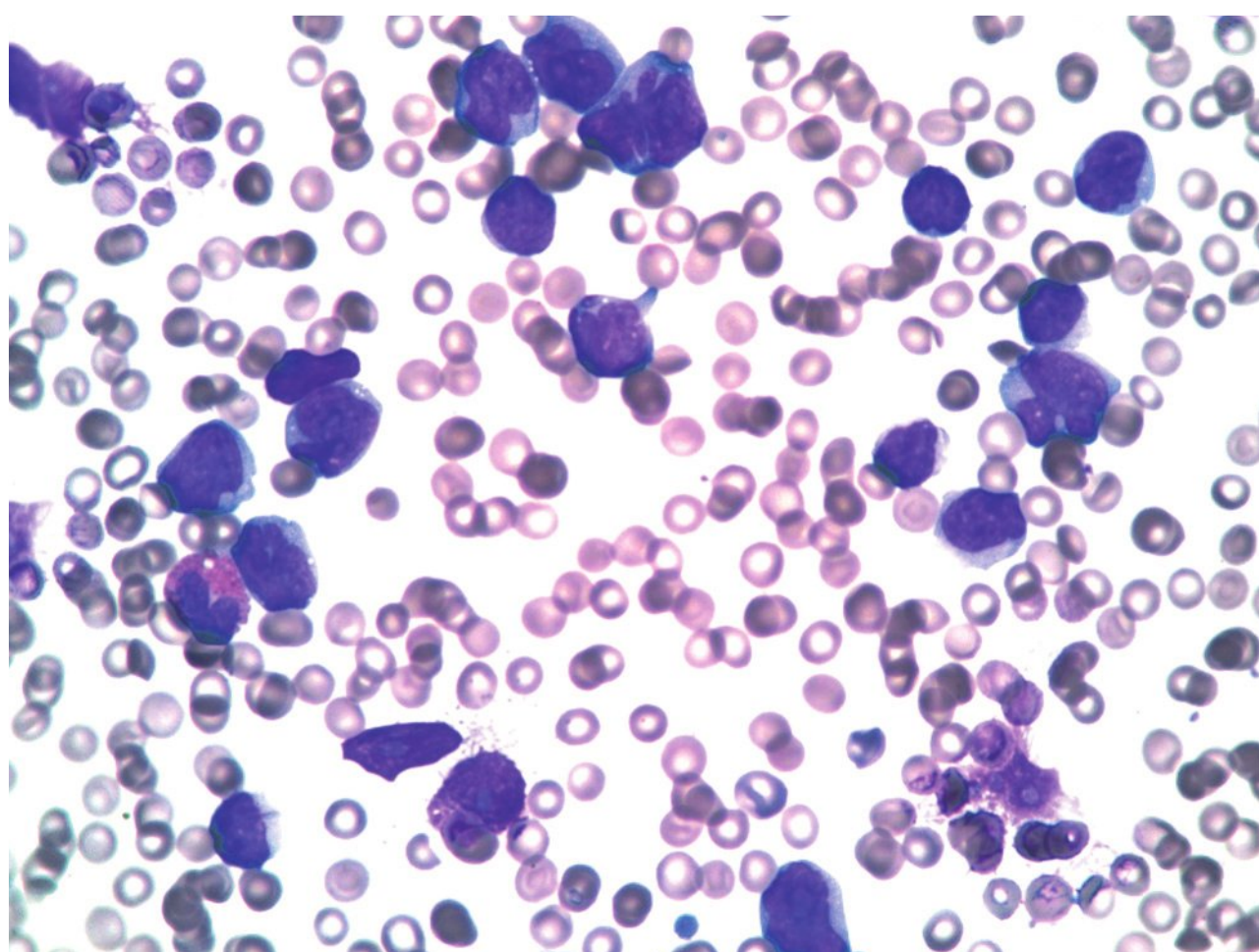


FIGURE 6.31.10 Peripheral blood smear shows many lymphoma cells with variable sizes, irregular configuration, high nuclear-cytoplasmic ratio, and prominent nucleoli. Wright-Giemsa stain, 60× magnification.

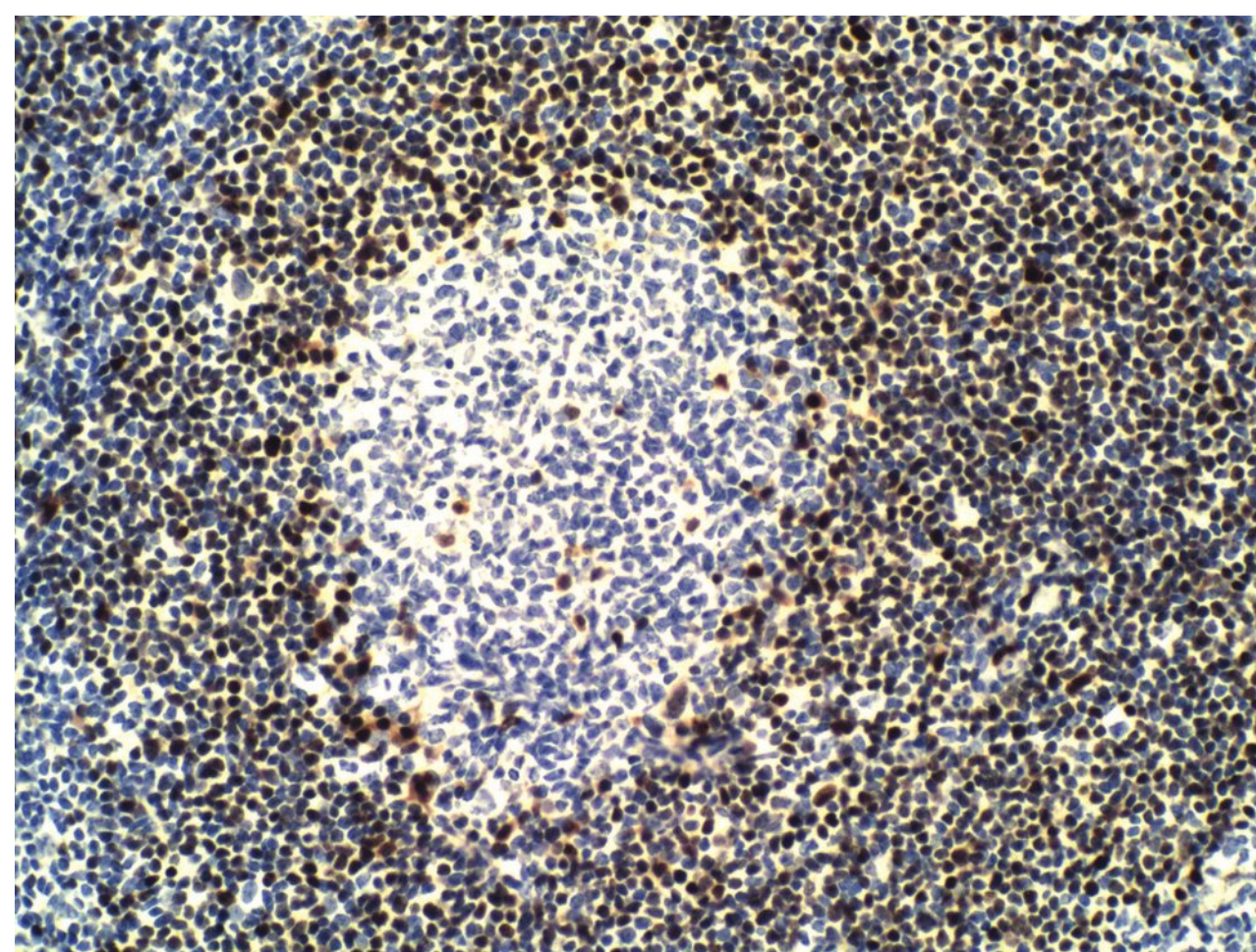


FIGURE 6.31.12 Lymph node biopsy of MCL case shows positive nuclear staining of cyclin D1 in the tumor cells located in the mantle zone. Immunoperoxidase, 20× magnification.

technique. A panel of CD5, CD20, CD23, CD79b/CD79a, and cyclin D1 staining on tissue sections often provides a reliable diagnosis. As mentioned before, a positive cyclin D1 staining is most reliable, but this staining was technically difficult (23,36). The new generation of antibodies usually provide reliable results (37).

However, a few true cyclin D1-negative MCL cases have been identified by gene expression profiling (GEP) and may be the result of replacement by cyclin D2 or D3 (8,38). Some of these cases showed t(2;12)(p12;p13), representing cyclin D2/k light-chain translocation (39). The cyclin D1-negative cases can also be identified by immunohistochemical demonstration of SOX11 protein (40). The clinicopathologic features and outcome of the cyclin D1-negative MCL patients as identified by SOX11 expression are similar to those of the cyclin D1-positive patients (40).

A high percentage of cyclin D1 in conjunction with Ki-67 and survivin staining also helps in predicting a poor prognosis. The identification of the blastoid variant of MCL is facilitated by the demonstration of cyclin D1, Ki-67, and cyclin-dependent kinase 4 (CDK4) on the tumor cells (41).

Molecular Genetics

MCL is characterized by the presence of t(11;14)(q13;q32), which can be detected in about 75% of cases by karyotyping (42). This represents the translocation of the proto-oncogene BCL-1(11q13) juxtaposed to the heavy-chain gene (14q32). A PRAD1 (parathyroid adenoma 1) or CCND1 gene is linked to the BCL-1 gene at its telomeric border. This gene encodes for cyclin D1, a cell-cycle regulatory protein. As a result of the translocation, the CCND1/cyclin D1 genes are deregulated. Therefore, the t(11;14)-carrying

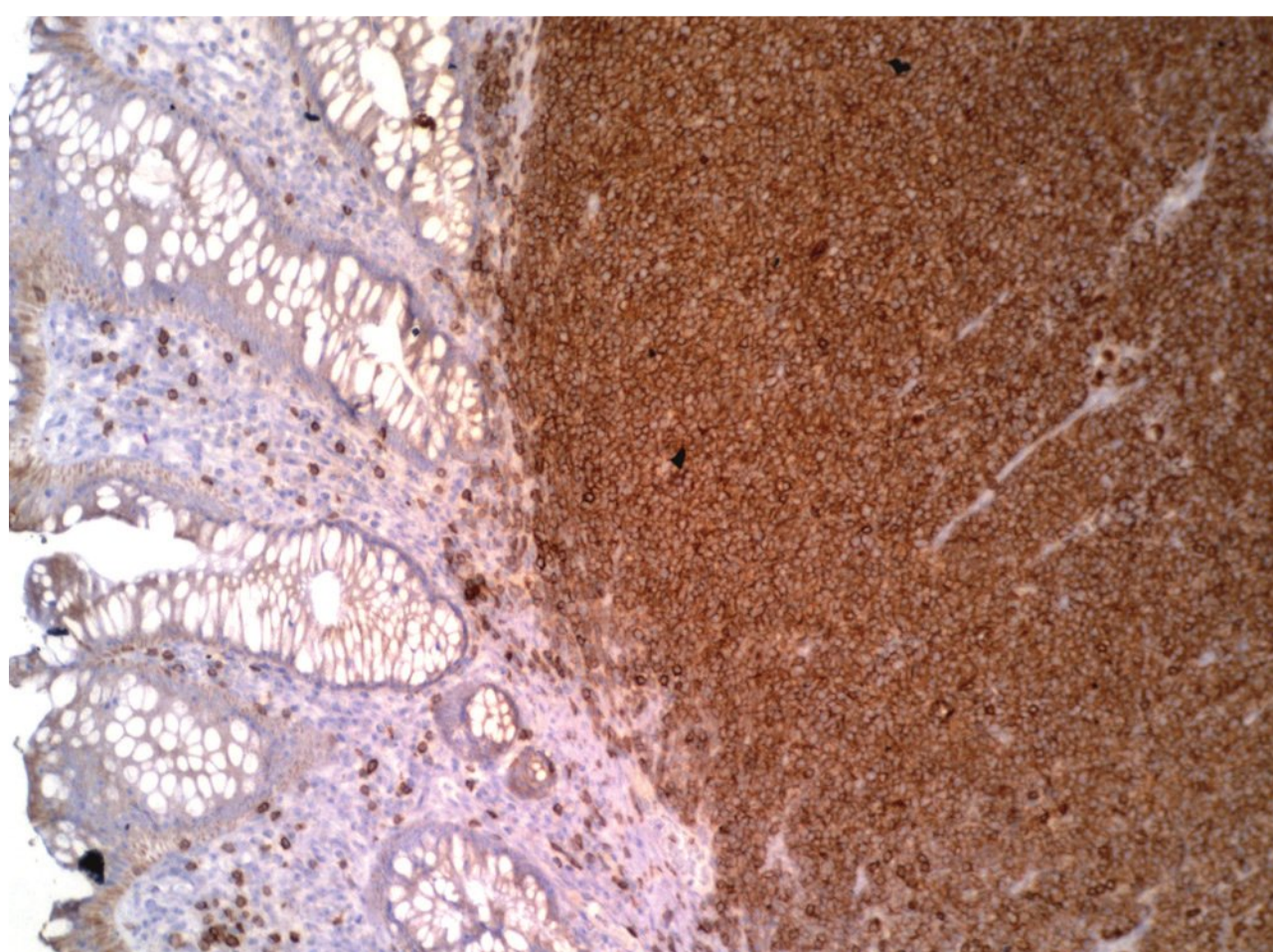


FIGURE 6.31.11 Colonic polyp reveals CD5 staining of 100% tumor cells. CD20 staining (not shown) demonstrates the same pattern. Immunoperoxidase, 10× magnification.

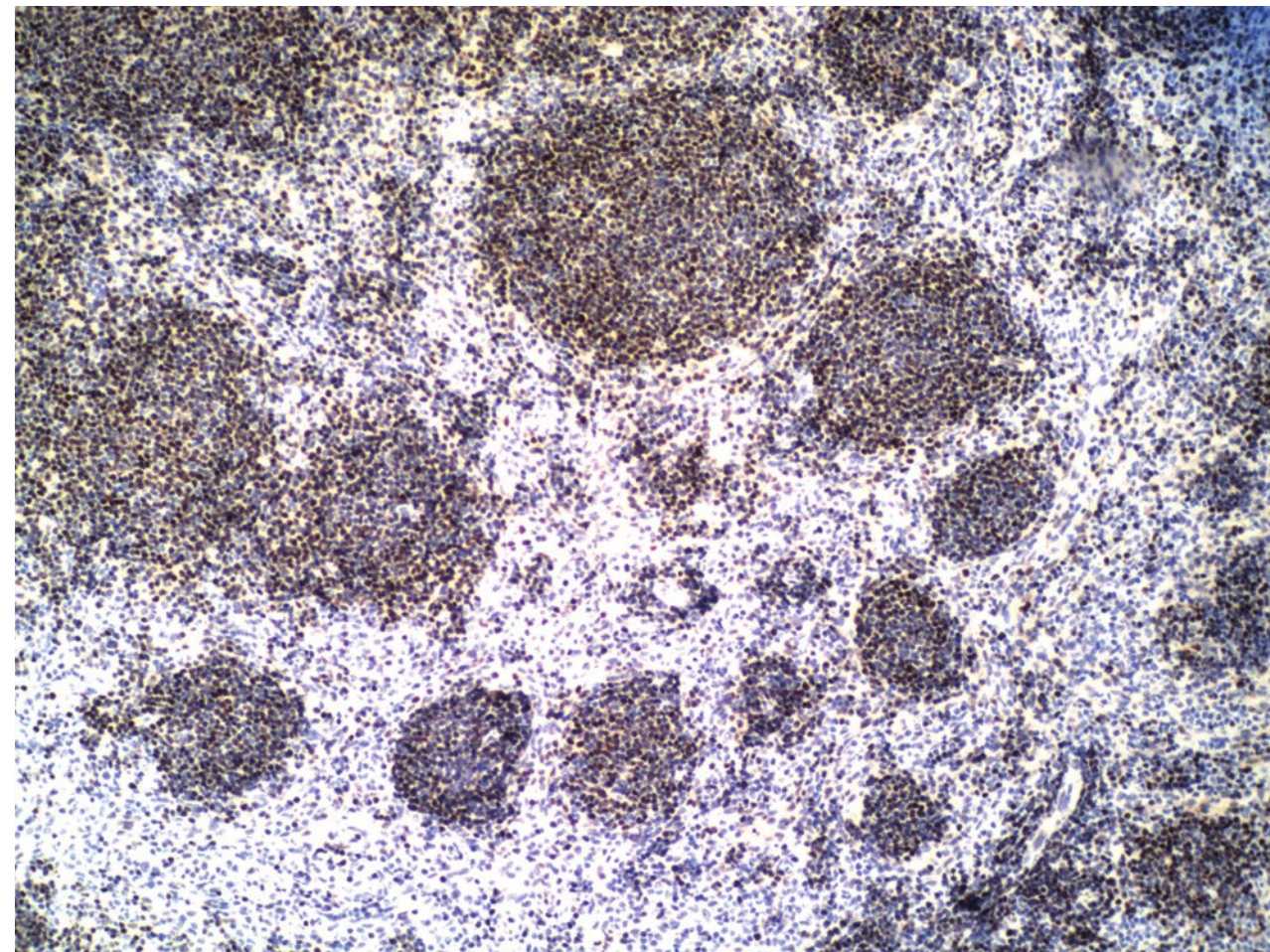


FIGURE 6.31.13 Splenectomy specimen shows multiple cyclin D1-positive nodules of varying sizes. Immunoperoxidase, 20× magnification.

cells cannot exit from the cell cycle, resulting in an expanded B-cell department with developmental arrest (14,43). However, the recent finding of the deletion of the ATM (ataxia telangiectasia mutated) gene in MCL suggests that ATM may serve as a tumor suppressor gene for MCL and that its deletion may be the mechanism leading to oncogenic transformation of the expanded B-cell population (44,45).

For the diagnosis of MCL, the detection of t(11;14) either by karyotyping or by molecular technique (Southern blot or polymerase chain reaction [PCR]) is highly specific, but generally the positive rate is not high. The low sensitivity of Southern blot and PCR is due to the large number of 11q13 breakpoints scattering over a region of >120 kb (42,46). The conventional karyotyping is hindered by the low yield and quality of tumor metaphases (42,46). Therefore, the method of choice for the detection of the translocation is the FISH technique using various probes (IgH/CNND1, CyclinD1/cep11, IgH/bcl-1).

According to the current literature, this technique is highly sensitive and specific for the diagnosis of MCL because the probes used cover a broad area so that t(11;14) can be detected irrespective of the location of the breakpoints within the BCL-1 region (23,42,46–49). In one study, only 4 of 32 non-MCL cases showed positive results by FISH (47). These false-positive results were found to be due to the presence of extra copies of chromosome 11 in 2 cases. Another report showed positive results in 11 of 21 “atypical” CLL cases with an MCL immunophenotype (47). These cases may well represent the leukemic phase of MCL. The additional advantage of FISH is that it can be performed on dried blood and bone marrow smears, fine-needle aspirates, and paraffin-embedded tissues.

The PRAD1/cyclin D1 messenger RNA can be detected by Northern blot or reverse transcriptase PCR (RT-PCR) (48,49). These techniques are sensitive for the diagnosis of MCL. RT-PCR, however, also showed cyclin D1 in 65% of non-MCL cases and 43% of atypical lymphoid hyperplasia cases in one study (49).

In addition to t(11;14), there have been many recurrent secondary karyotypic abnormalities reported (50). A few of them are of particular interest because of their possible association with particular genes. For instance, del 17p13 may be associated with p53, del 11q22-23 with ATM, and del 9p21 with p16/p15/p14. In contrast, amplification of 8q22-24 is associated with MYC and amplification of 10p12 is associated with the BMI-1 gene. BCL-6 gene is not amplified in MCL cases (50). The gain of BCL-2 gene in MCL is controversial (50,51). The blastoid variant is frequently associated with p53 mutation, p16 deletion, and tetraploidy (18,41,52).

It is hypothesized that the pathogenesis of MCL is essentially the result of dysregulation of the cell cycle through complex cytogenetic events. These include cyclin D1 upregulation, genomic amplification CDK4, deletions of the CDK inhibitor p16^{INK4a}, and overexpression of BMI-1 (53). The action of cyclin D1 is mainly through the binding of CDK4/CDK6, which phosphorylate and inactivate RB1 (tumor suppressor gene), leading to the release of E2F transcription factors and the subsequent progression of the

tumor cells from G1 to S phase (6,54). ATM and TP53 gene mutations are also involved in the pathogenesis of MCL (6).

GEP is able to stratify MCL cases into various subtypes in terms of IgH gene mutation status, proliferation rate, and blastoid morphology (55). GEP may also help to distinguish MCL from SLL, diffuse large B-cell lymphoma, and splenic marginal zone lymphoma (38,56). One GEP study identified a B-cell-associated tyrosine kinase gene, SYK, which was also demonstrated at RNA and protein levels in MCL cells (51). This gene serves as a possible therapeutic target in MCL and SYK inhibitor suppresses tumor cell proliferation and induces apoptosis in tumor cell lines.

In recent years, microRNA (miRNA) expression profiling has become a powerful tool for the study of MCL. miRNA is a class of 22-nucleotide noncoding RNA and deregulated miRNAs function as either oncogenes or tumor suppressors (54). As mentioned before, cyclin D1 overexpression exerts its function through activation of CDK4/CDK6. One study showed that miR-29 family acted as a tumor suppressor miRNA and inhibited CDK6 (54). In MCL cases, miR29 is downregulated and it may cooperate with cyclin D1 in MCL pathogenesis (54). Another study demonstrated that the lack of miR-20b expression was associated with favorable prognosis in MCL cases (57). The survival probability at 60 months in cases with high levels of miR-20b versus those without miR-20b was 33% and 56%, respectively (57). A third study of miRNA expression profiling identified two MCL tumor clusters characterized by different mutational status of the immunoglobulin (Ig) genes, proliferation signature, and number of genomic alterations (58).

As mentioned before, most MCL cases express surface light chain, which correlates well with the gene rearrangement studies that show the deletion of the k light-chain gene or rearrangement between the k deletion element and the recombinant signal sequence (50,59).

The vast majority of MCL cases, with the possible exception of blastoid variant, show unmutated Ig heavy-chain variable (V_H) gene, suggesting that the tumor cells are derived from naïve B cells (50,55). However, approximately 25% of MCL cases have mutated V_H genes. One study found that MCL cases with mutated status were associated with the absence of lymphadenopathy and good prognosis (60). However, most studies revealed no significantly different outcomes between mutated and unmutated cases (50,55). Unlike CLL cases, unmutated cases are not associated with Zeta chain-associated protein kinase (ZAP)-70 expression. The salient features for laboratory diagnosis of MCL are summarized in Table 6.31.3.

Clinical Manifestations

MCL is seen in elderly patients with a median age of about 60 to 63 years, and the male-to-female ratio ranges from 2.3:1 to 5:1 (7,13,59). The incidence of MCL in the United States is 2.5% to 4.0% of all non-Hodgkin lymphomas, but it is 7% to 9% in Europe.

Most (73%) patients present with generalized lymphadenopathy, and about 50% of patients have systemic symptoms (5.8%). About 50% of patients have splenomegaly at presentation, and 80% of patients with mantle zone subtype of MCL may show prominent splenomegaly. Most

TABLE 6.31.3

Salient Features for Laboratory Diagnosis of MCL

1. Monoclonal surface immunoglobulin pattern with intermediate staining intensity
2. Positive B-cell antigens: CD19, CD20, CD22, CD79b, HLA-DR
3. Characteristic marker for diagnosis: CD5
4. Important negative B-cell antigen: CD23
5. Characteristic cytogenetic abnormality: t(11;14) (q13;q32)
6. Molecular characterization: bcl-1 (CNND1)/IgH rearrangement
7. Nuclear cyclin D1 identified by immunohistochemistry or fluorescence in situ hybridization

patients with MCL are in stage III or IV at diagnosis, and bone marrow and liver involvement are common findings. Hepatomegaly is seen in 20% of patients. Primary extranodal presentation was considered an infrequent finding, but a report showed an incidence of 25% (60). The extranodal presentation is most commonly seen in the gastrointestinal tract and Waldeyer ring (14). Peripheral lymphocytosis of $>4,000/\text{mL}$ occurs in 20% to 40% of cases but seldom exceeds $20,000/\mu\text{L}$ (14). Mild anemia and thrombocytopenia are seen in some cases. Hypogammaglobulinemia, monoclonal gammopathy, and positive Coombs test have been occasionally reported.

MCL has the worst prognosis among all B-cell lymphomas, because it assumes an aggressive clinical course and yet is incurable. In terms of prognosis, most studies emphasize the association with the histologic subtypes, the blastoid morphology, and the cell markers. GEP studies support the stratification based on the above factors. One study found that del 8p21 and 13q14 were associated with inferior survival, whereas del 9p/p15-p16 showed a trend for decreased overall survival (61). Unlike CLL cases, the mutation status of the V_H gene does not predict the prognosis in MCL patients.

Clinically, several factors appear to be adverse prognostic indicators, including leukemic presentation, bone marrow involvement, advanced stage disease, B symptoms, and poor performance status (19,60,62,63).

The relationship between histologic types and prognosis is controversial. Weisenburger and Armitage (14) suggested that nodular and mantle zone types with a median survival of >5 years should be considered a low-grade lymphoma; the diffuse type with a median survival of 3 years, an intermediate-grade lymphoma; and the blastoid variant with a median survival of <2 years, a high-grade lymphoma. Majlis et al. (64) found that the 3-year survival rates were 100%, 50%, and 55% for patients with mantle zone, nodular, and diffuse histologic patterns, respectively. In contrast, Argatoff et al. (60) failed to show any significant differences in the median overall survival among the mantle

zone, nodular, and diffuse types. However, the more recent study by the Nebraska group showed that the diffuse type had the shortest median overall survival of 16 months, as compared to 55 months in the nodular type and 50 months in the blastoid variant (65).

The general consensus is that the blastoid variant is unequivocally associated with the worst prognosis (17,19,41,60,66). As mentioned before, the blastoid variant presents with high mitotic rates, and expresses with cyclin D1, Ki-67, CKD4, TP53 mutation, and p16 deletion. The blastoid variant is also characterized by the presence of extra copies of CCND1 signals (41). High levels of lactate dehydrogenase ($>450 \text{ U/L}$) and/or leukocytosis ($>10,000/\text{mL}$), high percentage of Ki-67, and high mitotic score at presentation are predictors for blastoid transformation (66). A recent study also showed that CKS-1B (CDC28 protein kinase regulatory subunit 1B), which is essential for the ubiquitination and degradation of p27 and cell cycle progression, had a much higher frequency of expression in blastoid/pleomorphic variants (87.5%) than in typical MCL cases (28.6%) (67).

On the other hand, there is a subgroup of MCL patients who present with an indolent clinical course (40,68). These patients are characterized by non-nodal presentation, predominantly hypermutated V_H gene, lack of genomic complexity, and absence of SOX11 expression (40,68). This group of patients has excellent clinical outcome and might be managed more conservatively than conventional MCL patients (40,68).

The current case is unusual for a relatively indolent clinical presentation for 9 years. It was until the development of blastoid transformation that an aggressive clinical course was followed, leading to a rapidly fatal outcome within 9 months. In the final months, the patient was found to have central nervous system, pulmonary, and abdominal involvement clinically, but the autopsy showed that almost all internal organs were involved, exemplifying a highly aggressive malignancy. Peghini and Fehr (21) reported an MCL patient similar to ours who survived for 19 years.

REFERENCES

1. Weisenburger DD. Mantle cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:789–801.
2. Lennert K, Feller AC. *B-Cell Lymphomas: Histopathology of Non-Hodgkin's Lymphomas (Based on the Updated Kiel Classification)*. 2nd ed. New York, NY: Springer-Verlag; 1992:93–102.
3. Banks PM, Chan J, Cleary ML, et al. Mantle cell lymphoma: a proposal for unification of morphologic, immunologic and molecular data. *Am J Surg Pathol*. 1992;16:637–640.
4. Harris NL, Jaffe ES, Stein H, et al. A Revised European-American Classification of Lymphoid Neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
5. Swerdlow SH, Berger F, Isaacson PI, et al. Mantle cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:168–170.

6. Swerdlow SH, Campo E, Seto M, et al. Mantle cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:229–232.
7. Isaacson PG, MacLennan KA, Subbuswamy SG. Multiple lymphomatous polyposis of the gastrointestinal tract. *Histopathology*. 1984;8:641–656.
8. Pileri SA, Falini B. Mantle cell lymphoma. *Haematological*. 2009;94:1488–1492.
9. Richard P, Vassallo J, Volmary S, et al. “In situ-like” mantle cell lymphoma: a report of two cases. *J Clin Pathol*. 2006;59:995–996.
10. Tiemann M, Schrader C, Klapper W, et al. Histopathology, cell proliferation indices and clinical outcome in 304 patients with mantle cell lymphoma (MCL): a clinicopathological study from the European MCL network. *Br J Haematol*. 2005;131:29–38.
11. Medeiros LJ, Jaffe ES. Low-grade B-cell lymphomas not specified in the working formulation. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. 2nd ed. Philadelphia, PA: W. B. Saunders; 1995:221–251.
12. Cohen PL, Kurtin PJ, Donovan KA, et al. Bone marrow and peripheral blood involvement in mantle cell lymphoma. *Br J Haematol*. 1998;101:302–310.
13. Wasman J, Rosenthal NS, Farhi DC. Mantle cell lymphoma: morphologic findings in bone marrow involvement. *Am J Clin Pathol*. 1996;106:196–200.
14. Weisenburger DD, Armitage JO. Mantle cell lymphoma—an entity comes of age. *Blood*. 1996;87:4483–4494.
15. Kumar S, Krenacs L, Orsuki T, et al. *bcl-1* rearrangement and cyclin D1 protein expression in multiple lymphomatous polyposis. *Am J Clin Pathol*. 1996;105:737–743.
16. Ruskone-Fourmestruix A, Delmer A, Lavergne A, et al. Multiple lymphomatous polyposis of the gastrointestinal tract: prospective clinicopathologic study of 31 cases. *Gastroenterology*. 1997;112:7–16.
17. Vallamudi G, Lionetti KA, Greenberg S, et al. Leukemic phase of mantle cell lymphoma: two case reports and review of the literature. *Arch Pathol Lab Med*. 1996;120:35–40.
18. Ott G, Kalla J, Ott M, et al. Blastoid variants of mantle cell lymphoma: frequent *bcl-1* rearrangements at the major translocation cluster region and tetraploid chromosome clones. *Blood*. 1997;89:1421–1429.
19. Kaleem Z, Wakoff AR, Smith RP, et al. Blastic transformation of mantle cell lymphoma. *Arch Pathol Lab Med*. 1996;120:577–580.
20. Singleton TP, Anderson MM, Ross CW, et al. Leukemic phase of mantle cell lymphoma, blastoid variant. *Am J Clin Pathol*. 1999;111:495–500.
21. Peghini PE, Fehr J. Analysis of cyclin D1 expression by quantitative real-time reverse transcription-polymerase chain reaction in the diagnosis of mantle cell lymphoma. *Am J Clin Pathol*. 2002;117:237–245.
22. Schlette E, Fu K, Medeiros LJ, et al. CD23 expression in mantle cell lymphoma: clinicopathologic features of 18 cases. *Am J Clin Pathol*. 2002;120:760–766.
23. Sun T, Nordberg ML, Cotelingam JD, et al. Fluorescence in situ hybridization: method of choice for a definitive diagnosis of mantle cell lymphoma. *Am J Hematol*. 2003;74:78–84.
24. Liu Z, Dong HY, Gorezyca W, et al. CD5– mantle cell lymphoma. *Am J Clin Pathol*. 2002;118:216–224.
25. Harris NL, Ferry JA. Follicular lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Lippincott Williams & Wilkins; 2001:823–854.
26. Somas AP, Matutes E, Morilla R, et al. Expression of the immunoglobulin-associated protein B29 in B-cell disorders with the monoclonal antibody, SN8 (CD79b). *Leukemia*. 1996;10:1966–1970.
27. Zukerberg LR, Yang WI, Arnold A, et al. Cyclin D1 expression in non-Hodgkin’s lymphomas: detection by immunohistochemistry. *Am J Clin Pathol*. 1995;103:756–760.
28. Taniguchi M, Oka K, Hiasa A, et al. De novo CD5+ diffuse large B-cell lymphomas express VH genes with somatic mutation. *Blood*. 1998;91:1145–1151.
29. Vasef MA, Medeiros LJ, Koo C, et al. Cyclin D1 immunohistochemical staining is useful in distinguishing mantle cell lymphoma from other low-grade B-cell neoplasms in bone marrow. *Am J Clin Pathol*. 1997;108:302–307.
30. Alkan S, Schnitzer B, Thompson JL, et al. Cyclin D1 protein expression in mantle cell lymphoma. *Ann Oncol*. 1995;6:567–570.
31. Yatabe Y, Nakamura S, Seto M, et al. Clinicopathologic study of PRAD1/cyclin D1 overexpressing lymphoma with special reference to mantle cell lymphoma. A distinct molecular pathologic entity. *Am J Surg Pathol*. 1996;20:1110–1122.
32. Yatabe Y, Suzuki R, Tobinai K, et al. Significance of cyclin D1 overexpression for the diagnosis of mantle cell lymphoma: a clinicopathologic comparison of cyclin D1-positive MCL and cyclin D1-negative MCL-like B-cell lymphoma. *Blood*. 2000;95:2253–2261.
33. Elnenaei ML, Jadayel DM, Matutes E, et al. Cyclin D1 by flow cytometry as a useful tool in the diagnosis of B-cell malignancies. *Leuk Res*. 2001;25:115–123.
34. Martinez A, Bellosillo B, Bosch F, et al. Nuclear surviving expression in mantle cell lymphoma is associated with cell proliferation and survival. *Am J Pathol*. 2004;164:501–510.
35. Hock BD, McKenzie JL, Patton NW, et al. Circulating levels and clinical significance of soluble CD40 in patients with hematologic malignancies. *Cancer*. 2006;106:2148–2157.
36. Campo E. Genetic and molecular genetic studies in the diagnosis of B-cell lymphomas I. Mantle cell lymphoma, follicular lymphoma, and Burkitt’s lymphoma. *Hum Pathol*. 2003;34:330–335.
37. Cheuk W, Wong KO, Wong CS, et al. Consistent immunostaining for cyclin D1 can be achieved on a routine basis using a newly available rabbit monoclonal antibody. *Am J Surg Pathol*. 2004;28:801–807.
38. Rosenwald A, Wright G, Wiestner A, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell*. 2003;3:185–197.
39. Gesk S, Klapper W, Martin-Subero JJ, et al. A chromosomal translocation in cyclin D1-negative/cyclin D2-positive mantle cell lymphoma fuses the CCND2 gene to the IGH locus. *Blood*. 2006;108:1109–1110.
40. Dreyling M, Hoster E, Bea S, et al. Update on the molecular pathogenesis and clinical treatment of mantle cell lymphoma (MCL): minutes of the 9th European MCL Network conference. *Leuk Lymphoma*. 2010;51(9):1612–1622.
41. Parrens M, Belaud-Rotureau MA, Fitoussi O, et al. Blastoid and common variants of mantle cell lymphoma exhibit distinct immunophenotypic and interphase FISH features. *Histopathology*. 2006;48:353–362.
42. Siebert R, Mathiesen P, Harder S, et al. Application of interphase cytogenetics for the detection of t(11;14)(q13;q32) in mantle cell lymphomas. *Ann Oncol*. 1998;9:519–526.
43. Rimokh R, Berger F, Delso G, et al. Detection of the chromosomal translocation t(11;14) by polymerase chain reaction in mantle cell lymphomas. *Blood*. 1994;83:1871–1875.
44. Stilgenbauer S, Schaffner C, Winkler D, et al. The ATM gene in the pathogenesis of mantle cell lymphoma. *Ann Oncol*. 2000;11(suppl 1):127–130.

45. Schaffner C, Idler I, Stilgenbauer S, et al. Mantle cell lymphoma is characterized by inactivation of the ATM gene. *Proc Natl Acad Sci U S A*. 2000;97:2773–2778.
46. Monteil M, Callanan M, Dascalescu C, et al. Molecular diagnosis of t(11;14) in mantle cell lymphoma using two-color interphase fluorescence in situ hybridization. *Br J Haematol*. 1996;13:797–802.
47. Avet-Loiseau H, Garand R, Gaillard F, et al. Detection of t(11;14) using interphase molecular cytogenetics in mantle cell lymphoma and atypical chronic lymphocytic leukemia. *Gen Chrom Cancer*. 1998;23:175–182.
48. de Boer CJ, Schuurin E, Dreef E, et al. Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. *Blood*. 1995;86:2715–2723.
49. Aguilera NS, Bijwaard KE, Duncan B, et al. Differential expression of cyclin D1 in mantle cell lymphoma and other non-Hodgkin's lymphomas. *Am J Pathol*. 1998;153:1969–1976.
50. Bertoni F, Zucca E, Cotter FE. Molecular basis of mantle cell lymphoma. *Br J Haematol*. 2004;124:130–140.
51. Rinaldi A, Kwee I, Tadorelli M, et al. Genomic and expression profiling identifies the B-cell associated tyrosine kinase Syk as a possible therapeutic target in mantle cell lymphoma. *Br J Haematol*. 2005;132:303–316.
52. Hernandez L, Fest T, Cazorla M, et al. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. *Blood*. 1996;87:3351–3359.
53. Fernandez V, Hartmann E, Ott G, et al. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. *J Clin Oncol*. 2005;23:6364–6369.
54. Zhou JJ, Lin J, Lwin T, et al. microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma. *Blood*. 2010;115:630–2639.
55. Bartoni F, Zucca E, Cavalli F. Mantle cell lymphoma. *Curr Opin Hematol*. 2004;11:411–418.
56. Thieblemont C, Nasser V, Felman P, et al. small lymphocytic lymphoma, marginal zone B-cell lymphoma, and mantle cell lymphoma exhibit distinct gene-expression profile allowing molecular diagnosis. *Blood*. 2004;103:2727–2737.
57. Di Lisio L, Gomez-Lopez G, Sanchez-Beato M, et al. Mantle cell lymphoma: transcriptional regulation by microRNAs. *Leukemia*. 2010;24:1335–1342.
58. Navarro A, Bea S, Fernandez V, et al. MicroRNA expression, chromosomal alterations, and immunoglobulin variable heavy chain hypermutations in mantle cell lymphomas. *Cancer Res*. 2009;69:7071–7978.
59. Bertoni F, Zucca E, Genini D, et al. Immunoglobulin light chain kappa deletion rearrangement as a marker of clonality in mantle cell lymphoma. *Leuk Lymphoma*. 1999;36:147–150.
60. Argatoff LH, Connors JM, Kiasa RJ, et al. Mantle cell lymphoma. A clinicopathologic study of 80 cases. *Blood*. 1997;89:2067–2078.
61. Williams ME, Densmore JJ. Biology and therapy of mantle cell lymphoma. *Curr Opin Oncol*. 2005;17:425–431.
62. Leonard JP, Schattner EJ, Coleman M. Biology and management of mantle cell lymphoma. *Curr Opin Oncol*. 2001;13:342–347.
63. Bosch F, Lopez-Guillermo A, Campo E, et al. Mantle cell lymphoma presenting features, response to therapy, and prognostic factors. *Cancer*. 1998;82:567–575.
64. Majlis A, Pugh WC, Rodriquez MA, et al. Mantle cell lymphoma: correlation of clinical outcome and biologic features with three histologic variants. *J Clin Oncol*. 1997;15:1664–1671.
65. Weisenburger DD, Vose JM, Greiner TC, et al. Mantle cell lymphoma. A clinicopathologic study of 68 cases from the Nebraska Lymphoma Study Group. *Am J Hematol*. 2000;64:190–196.
66. Raty R, Franssila K, Jansson SE, et al. Predictive factors for blastoid transformation in the common variant of mantle cell lymphoma. *Eur J Cancer*. 2003;39:321–329.
67. Akyurek N, Drakos E, Giaslakitios K, et al. Differential expression of CKS-1B in typical and blastoid variants of mantle cell lymphoma. *Hum Pathol*. 2010;41(10):1448–1455.
68. Fernandez V, Salamero O, Espinet B, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. *Cancer Res*. 2010;70:1408–1418.

CASE 32

Diffuse Large B-Cell Lymphoma

CASE HISTORY

A 65-year-old man was admitted to the hospital for splenectomy. The patient noticed a mass in his left upper quadrant 7 months prior to admission, and the mass appeared to progress in the last 4 months. He denied having any constitutional symptoms such as fever, night sweats, and/or weight loss. A fine-needle aspiration was performed on an axillary lymph node, which showed only reactive cells with no evidence of lymphoma.

Laboratory examination revealed a total leukocyte count of 8,400/mL with 27% neutrophils, 58.1% lymphocytes,

13.2% monocytes, 1.4% eosinophils, and 0.3% basophils. The hematocrit was 48%, hemoglobin 16.3 g/dL, and platelets 141,000/mL. Serum lactate dehydrogenase was 159 IU/L.

During his hospital stay, a diagnosis of diffuse large B-cell lymphoma (DLBCL) was made on the splenectomy specimen. The patient was started with chemotherapy and continued to receive eight cycles of CHOP in the subsequent years. Since then, he had been in complete remission for 5 years with one bout of pneumonia and one episode of deep venous thrombosis during this period of time. However, an enlarged left axillary lymph node was discovered on a follow-up examination. A biopsy of the

lymph node was taken, and the diagnosis proved to be recurrent DLBCL.

The patient was treated with fludarabine without effect as the lymphoma spread to the cervical, thoracic, and lumbar spine. He was switched to another chemotherapeutic regimen as well as radiation therapy with resultant partial remission. However, the patient developed postradiation esophagitis, and his condition deteriorated rapidly. He died approximately 6 years after the initial diagnosis of lymphoma.

FLOW CYTOMETRY FINDINGS

Lymph node: CD5 3%, CD19 94%, CD20 98%, CD10 4%, CD23 1%, FMC-7 98%, κ 86%, λ 0%, CD19/ κ 87%, CD19/ λ 5%, CD45 100% (Fig. 6.32.1).

IMMUNOHISTOCHEMICAL STAINS

Lymph node: Positive reactions were demonstrated for CD20 and CD30 (Fig. 6.32.2), but negative reactions for CD3 and bcl-2.

MOLECULAR GENETICS

Lymph node: 46,XY, del(6)(q15),del(13)(q12q14), add(14)(q32) [9]/46,XY[7]. Fluorescence in situ hybridization for MYC oncogene was negative in all 20 interphase cells analyzed.

DISCUSSION

DLBCL is a non-Hodgkin lymphoma of B-cell origin showing diffuse proliferation of large neoplastic lymphoid cells. A large cell is defined by the World Health Organization (WHO) classification and others as having a nucleus equal to or exceeding normal macrophage nuclei or more than twice the size of a normal lymphocyte (1). As will be discussed later, this lymphoma group is morphologically heterogeneous and can be divided into several variants with minor differences in immunophenotypes but having similar clinical outcomes.

In the Working Formulation, DLBCL includes DLBCL and immunoblastic lymphoma (2). The Revised European-American Lymphoma (REAL) classification includes all aggressive large B-cell lymphomas (LBCLs) other than

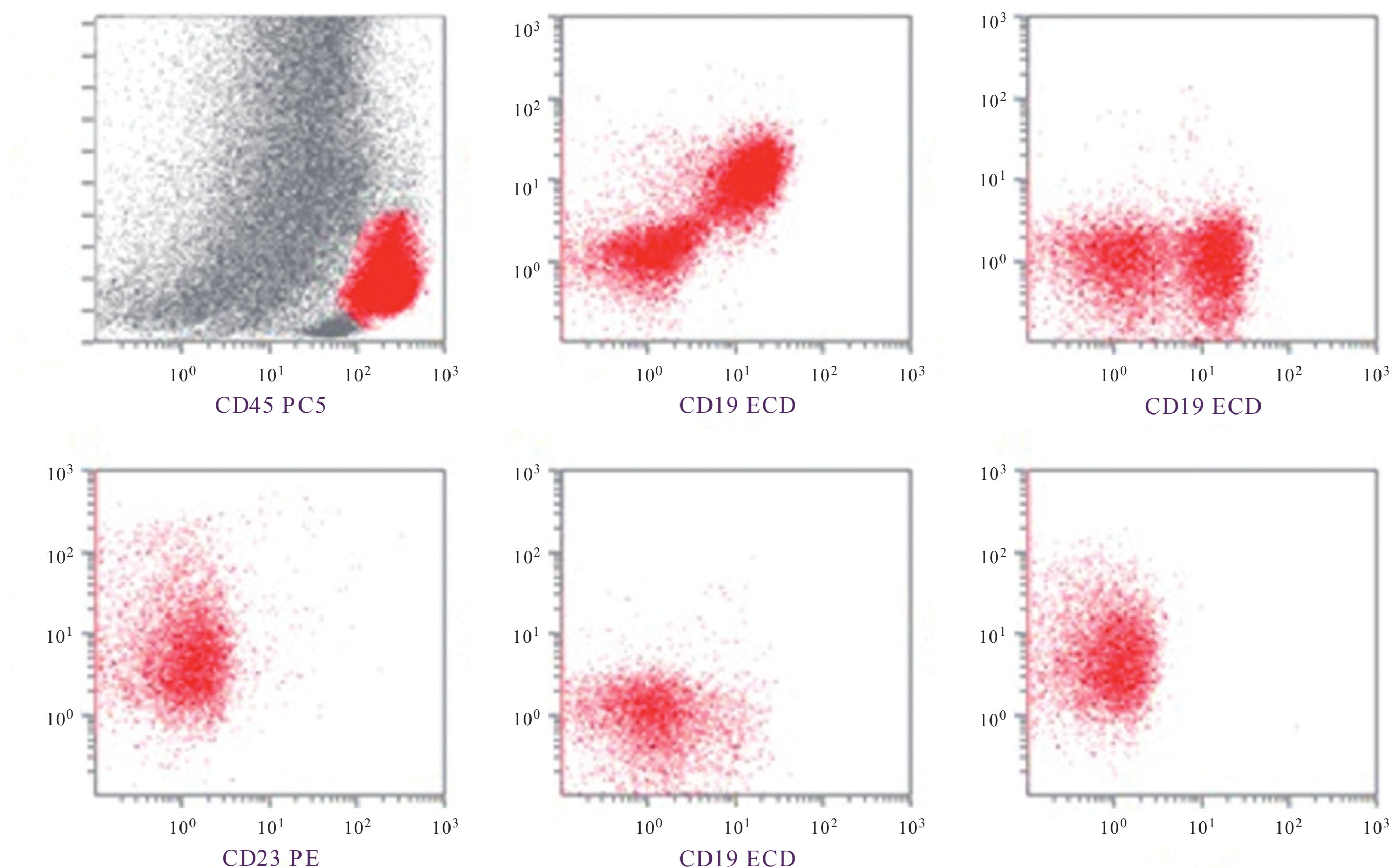


FIGURE 6.32.1 Flow cytometric histograms show positive CD19, CD20, κ , and FMC-7 but negative CD5, CD23, and λ reactions. SS, side scatter; PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; ECD, phycoerythrin-Texas Red; PE, phycoerythrin.

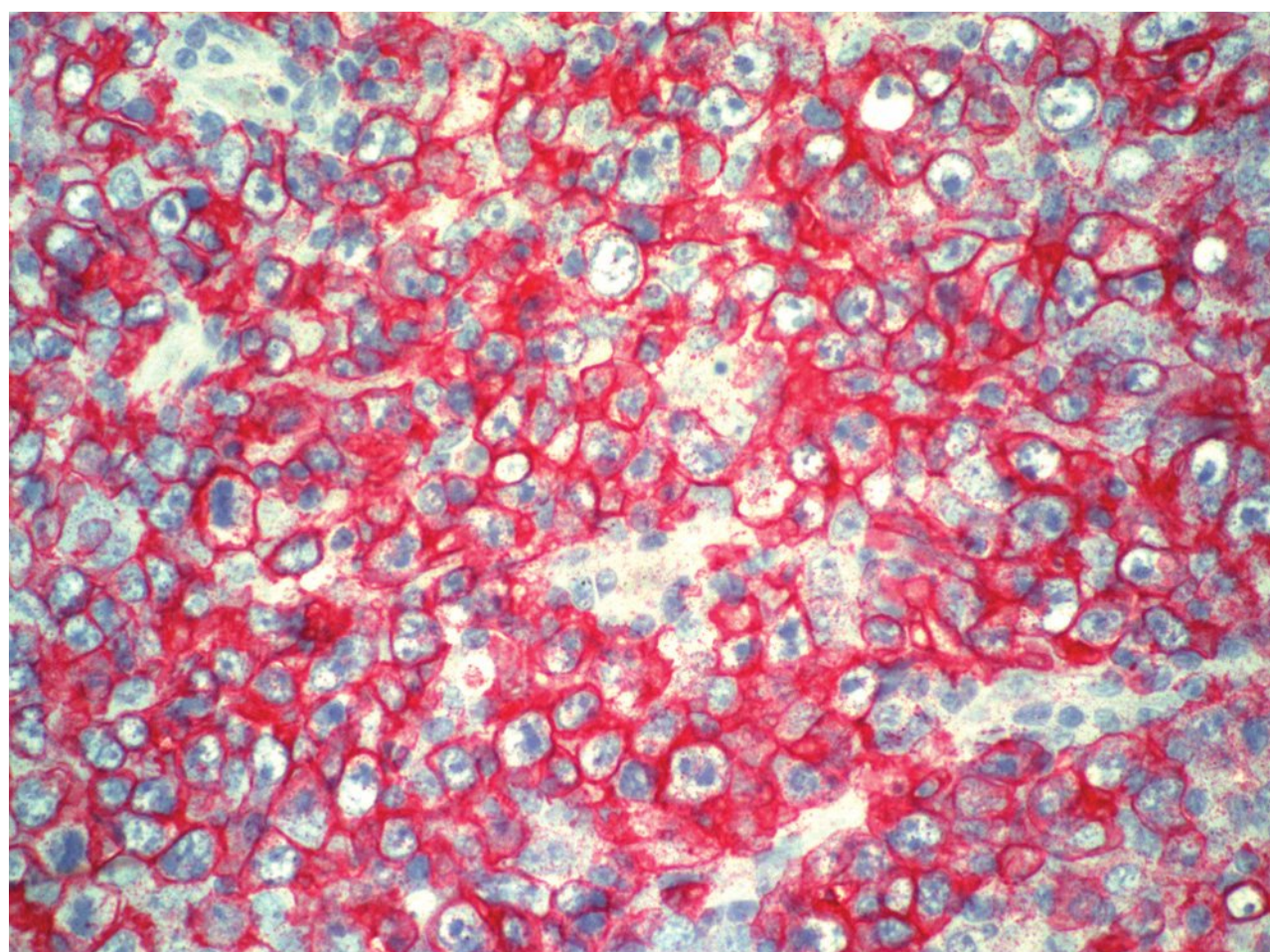


FIGURE 6.32.2 Lymph node biopsy shows positive staining of CD30 on the large tumor cells. Immunoalkaline phosphatase, 40× magnification.

lymphoblastic lymphoma and Burkitt lymphoma in the category of DLBCL (3). However, because primary mediastinal B-cell lymphoma differs from other DLBCL variants, it stands out as a special subtype of DLBCL in the REAL classification.

In the 2008 WHO classification, the scope of DLBCL is greatly expanded (Table 6.32.1) (4). Its morphologic subtypes include T-cell/histiocyte-rich LBCL; primary DLBCL of the central nervous system; primary cutaneous DLBCL, leg type; and Epstein–Barr virus (EBV)-positive DLBCL of the elderly. Cases not belonging to the above subtypes are designated DLBCL, not otherwise specified (NOS). The category of lymphomas of large cells includes primary mediastinal (thymic) LBCL, intravascular LBCL, DLBCL associated with chronic inflammation, lymphomatoid granulomatosis, ALK-positive DLBCL, plasmablastic lymphoma, LBCL arising in HHV8-associated multicentric Castleman disease, and primary effusion lymphoma. In addition, there are two borderline cases, which include B-cell lymphomas intermediate between DLBCL and Burkitt lymphoma, and B-cell lymphoma intermediate between DLBCL and classical Hodgkin lymphoma.

DLBCL is one of the most common lymphomas, accounting for 25% to 30% of adult non-Hodgkin lymphoma in Western countries (4), and 7 per 100,000 person-years in the United States (5). It can be de novo or secondary, being transformed from low-grade lymphomas, such as small lymphocytic lymphoma, follicular lymphoma, lymphoplasmacytic lymphoma, extranodal marginal zone B-cell lymphoma, and splenic marginal zone lymphoma.

Morphology

There are three common morphologic variants and several rare variants recognized by the WHO system. The rare variants with myxoid stroma, fibrillary matrix, pseudorosettes, spindly cells, signet ring cells, cytoplasmic granules, microvillous projections, and intercellular junction are not listed as distinct entities (1,4). The histologic pattern is characterized by a diffuse large-cell infiltration with effacement of the

TABLE 6.32.1

WHO Categorization of Diffuse Large B-Cell Lymphoma (DLBCL)

DLBCL, NOS

Common morphologic variants

Centroblastic

Immunoblastic

Anaplastic

Rare morphologic variants

Molecular subgroups

Germinal center B-cell-like (GCB)

Activated B-cell-like (ABC)

Immunohistochemical subgroups

CD5-positive DLBCL

Germinal center B-cell-like (GCB)

Non-germinal center B-cell-like (non-GCB)

DLBCL subtypes

T-cell/histiocyte-rich LBCL

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

EBV positive DLBCL of the elderly

Other lymphomas of large B cells (LBCL)

Primary mediastinal (thymic) LBCL

Intravascular LBCL

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

ALK-positive LBCL

Plasmablastic lymphoma

LBCL arising in HHV8 associated multicentric Castleman disease

Primary effusion lymphoma

Borderline cases

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and Burkitt lymphoma

B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical Hodgkin lymphoma

normal architecture, but it can also be interfollicular or intrasinusoidal infiltration. It is recognized that these variants are not easily reproducible between different observers.

Centroblastic Variant

This variant is composed of tumor cells similar to the centroblasts seen in reactive germinal centers. These cells vary from medium to large size and are characterized by large round or oval nuclei with a vesicular chromatin pattern and two to four membrane-bound nucleoli (Fig. 6.32.3) (1,4,6).

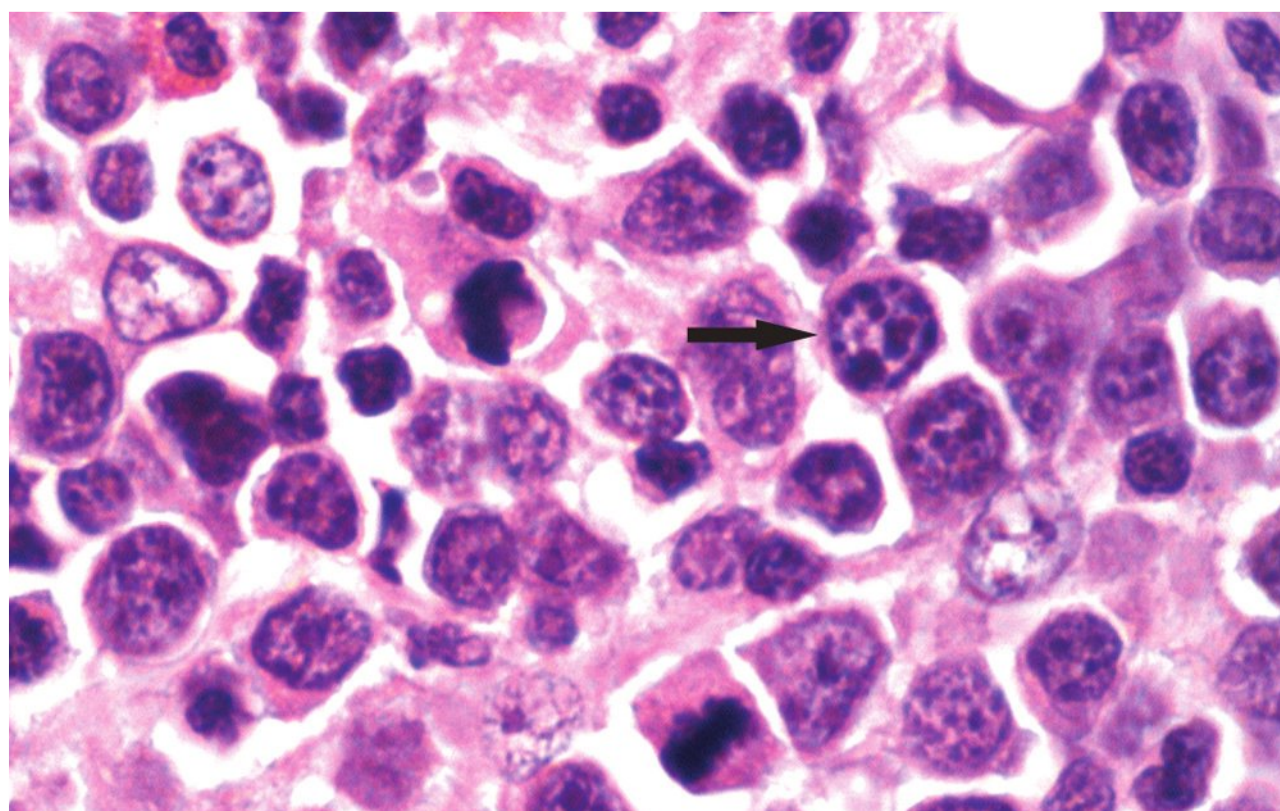


FIGURE 6.32.3 Centroblastic variant of DLBCL in a lymph node biopsy. Tumor cells show large round or oval nuclei with multiple membrane-bound nucleoli (arrow) and scanty cytoplasm. Hematoxylin and eosin, 100× magnification.

The cytoplasm is scanty and amphophilic to basophilic. This variant can be further divided into monomorphic, pleomorphic, and multilobated subvariants. The monomorphic type is composed of predominantly centroblasts. The pleomorphic type comprises a mixed population of centroblasts, immunoblasts, and/or multilobated cells. When pleomorphic type has more than 90% of immunoblasts, it is difficult to distinguish pleomorphic from immunoblastic variant. The multilobated type shows cells with the nuclei composed of more than three lobes and usually inconspicuous nucleoli (6). This variant is the most common among the variants.

Immunoblastic Variant

This variant encompasses >90% of immunoblasts (1,4,6). The immunoblasts are large cells with large vesicular nuclei and a single prominent, centrally located nucleolus (Fig. 6.32.4). There is a moderate amount of basophilic

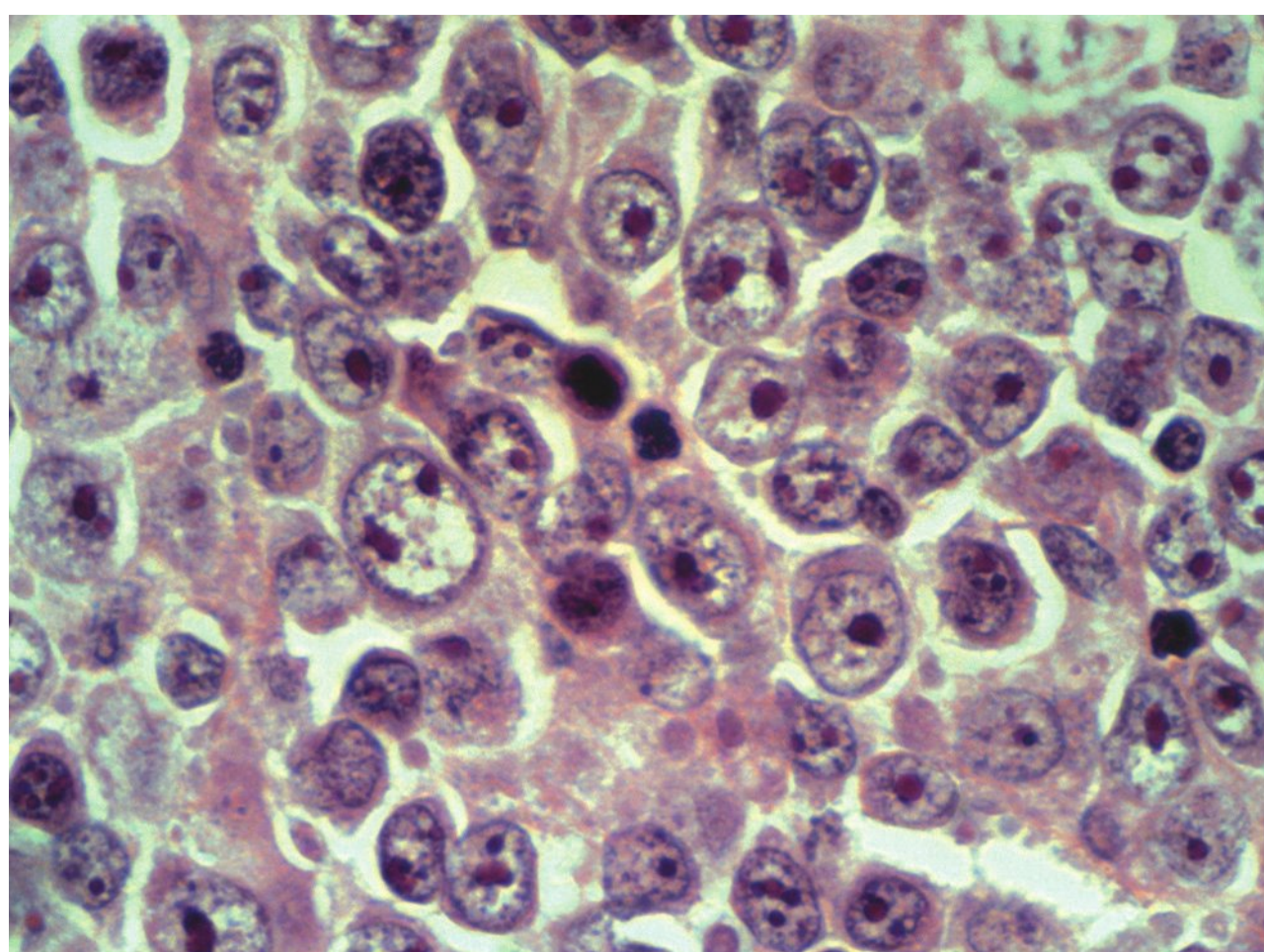


FIGURE 6.32.4 Immunoblastic variant of DLBCL in a lymph node biopsy. Tumor cells show large vesicular nuclei with single prominent nucleolus and moderate amount of cytoplasm. Hematoxylin and eosin, 100× magnification.

cytoplasm. Centroblasts, if present, should be <10% of the population, or it should be classified as a centroblastic variant. Plasma cells and plasmablasts may be seen in this variant. The immunoblastic variant accounts for only 4% of non-Hodgkin lymphomas in the Kiel classification (7).

Anaplastic Variant

This variant is characterized by a proliferation of large, anaplastic, and bizarre neoplastic cells (Fig. 6.32.5) (1,4,6). It is similar to anaplastic large lymphoma of T-cell or null cell origin not only morphologically but also in the positive staining with CD30 antigen. However, this variant usually does not have the same molecular cytogenetic characteristics of the T-cell/null cell type of anaplastic large-cell lymphoma, so they are considered biologically different. In addition, B-cell anaplastic variant is clinically more similar to other variants of DLBCL; thus, it is classified under this entity. The anaplastic variant may show a cohesive growth or a sinusoidal infiltration pattern and should be distinguished from metastatic carcinoma by immunophenotyping.

There are two old variants that require immunophenotyping to distinguish them from the above variants. In the 2008 WHO classification, they become independent entities.

1. Plasmablastic lymphoma (Fig. 6.32.6): This lymphoma has a morphologic spectrum varying from immunoblast-like to plasma cell-like. Mitosis is prominent and apoptosis may also be present. EBV infection is demonstrated by in situ hybridization in 60% to 75% of patients (8). Plasmablastic lymphoma is difficult to distinguish from DLBCL in some cases or from plasma cell myeloma from others on morphologic basis. However, its clinical presentation of an oral lesion in human immunodeficiency virus-infected patients frequently gives the clue to this diagnosis (8–10). This lymphoma may also be seen in other immunodeficiency diseases or in elderly people without obvious immunodeficiency. It also occurs in

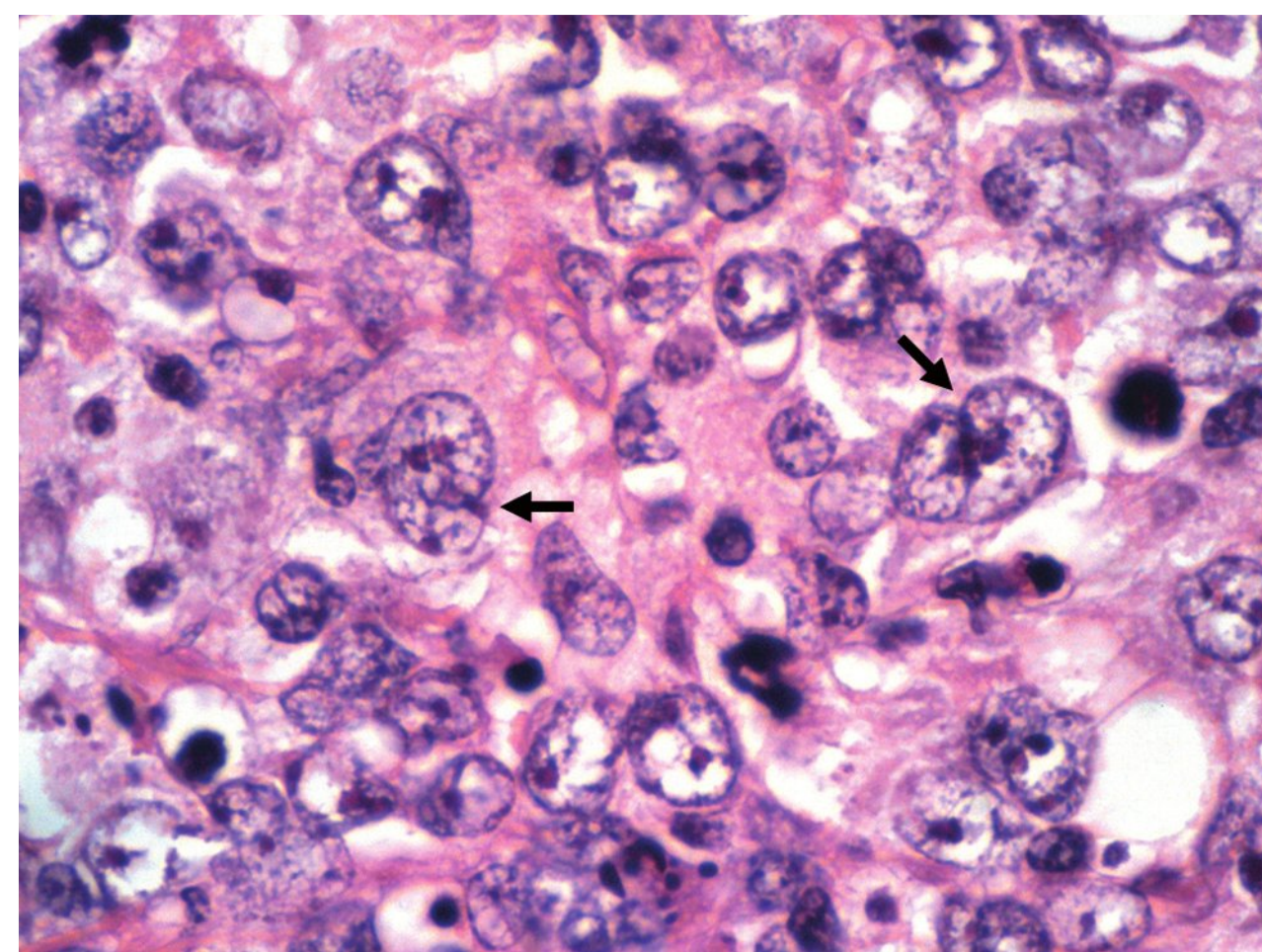


FIGURE 6.32.5 Anaplastic variant of DLBCL in a lymph node biopsy shows large anaplastic tumor cells with vesicular nuclei and prominent nucleoli. There are two characteristic kidney-shaped nuclei in the center of this field (arrows). Hematoxylin and eosin, 100× magnification.

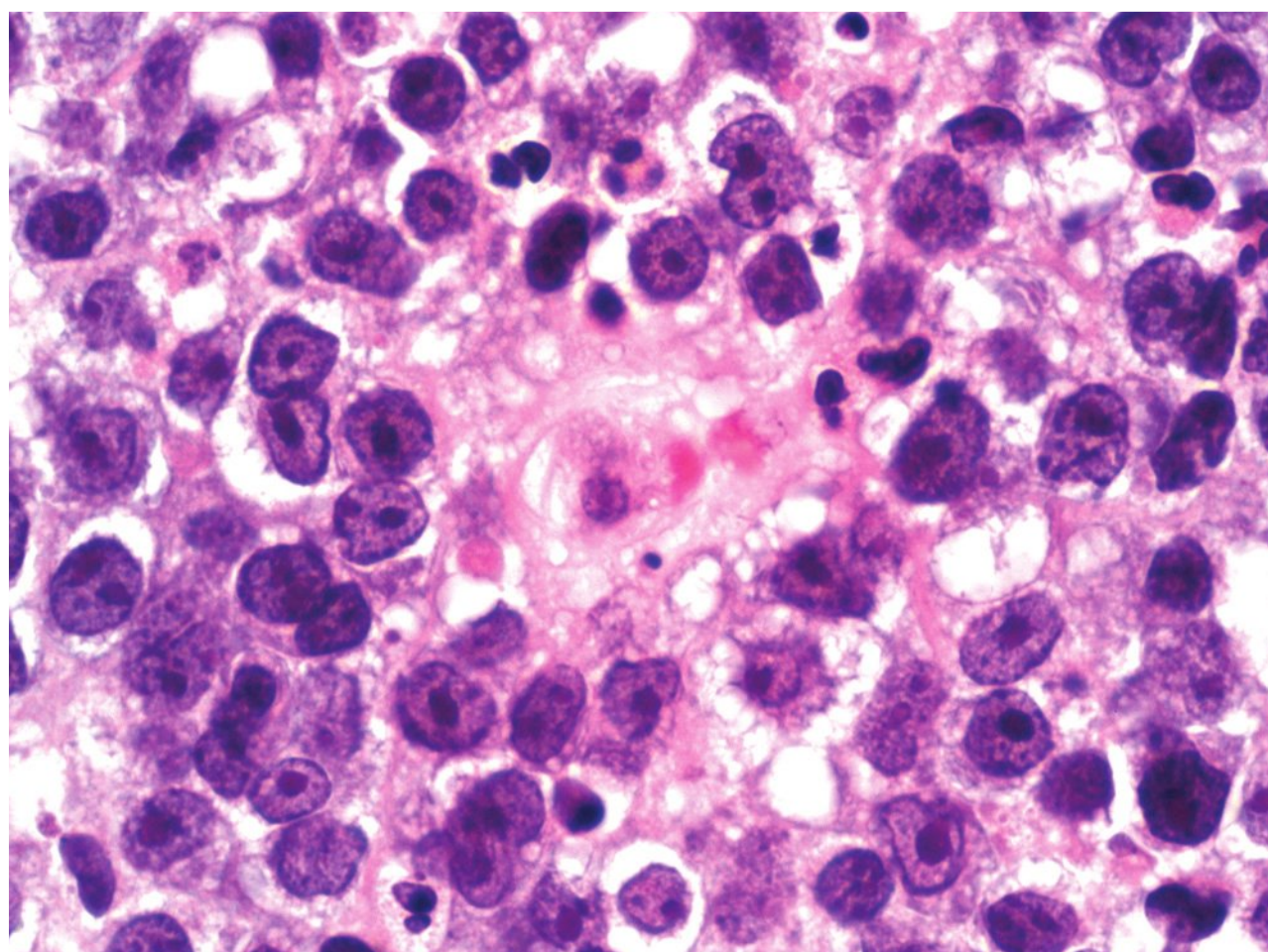


FIGURE 6.32.6 Plasmablastic lymphoma from a rectal biopsy shows many large tumor cells with vesicular nucleus and a single prominent nucleolus in most cells, mimicking immunoblasts. Hematoxylin and eosin, 100× magnification.

other extranodal sites, including the sinonasal cavity, orbit, skin, bone, soft tissues, and gastrointestinal tract (8). Lymph node involvement is rare. The immunophenotype is closer to that of plasmacytoma than to that of DLBCL (6). The tumor cells are probably in the differentiation stage between a B-immunoblast and a plasma cell.

2. ALK-positive LBCL: This lymphoma comprises monomorphic immunoblast-like cells but can also encompass plasmablasts in some cases (11,12). Histologically, a prominent sinusoidal infiltration pattern is frequently demonstrated. Its unique feature is the expression of ALK protein with granular cytoplasm and the Golgi staining pattern. The ALK gene can also be demonstrated by polymerase chain reaction (PCR) and the most frequent karyotype is t(2;17)(p23;q23) (11).

The characteristic morphologic features of DLBCL are summarized in Table 6.32.2.

Immunophenotype

DLBCL expresses all B-cell markers, including CD19, CD20 (Fig. 6.32.7), CD22, CD79a, and surface immunoglobulin (Ig). Cytoplasmic Ig is demonstrated in cases when plasmacytic differentiation is present. In a subset of DLBCL,

TABLE 6.33.2

Characteristic Morphologic Features of DLBCL

Histologic pattern	Diffuse large-cell infiltration, can be interfollicular or sinusoidal
Cytology	Centroblastic, immunoblastic, or anaplastic
Specific feature	Combination of histologic pattern and cytology

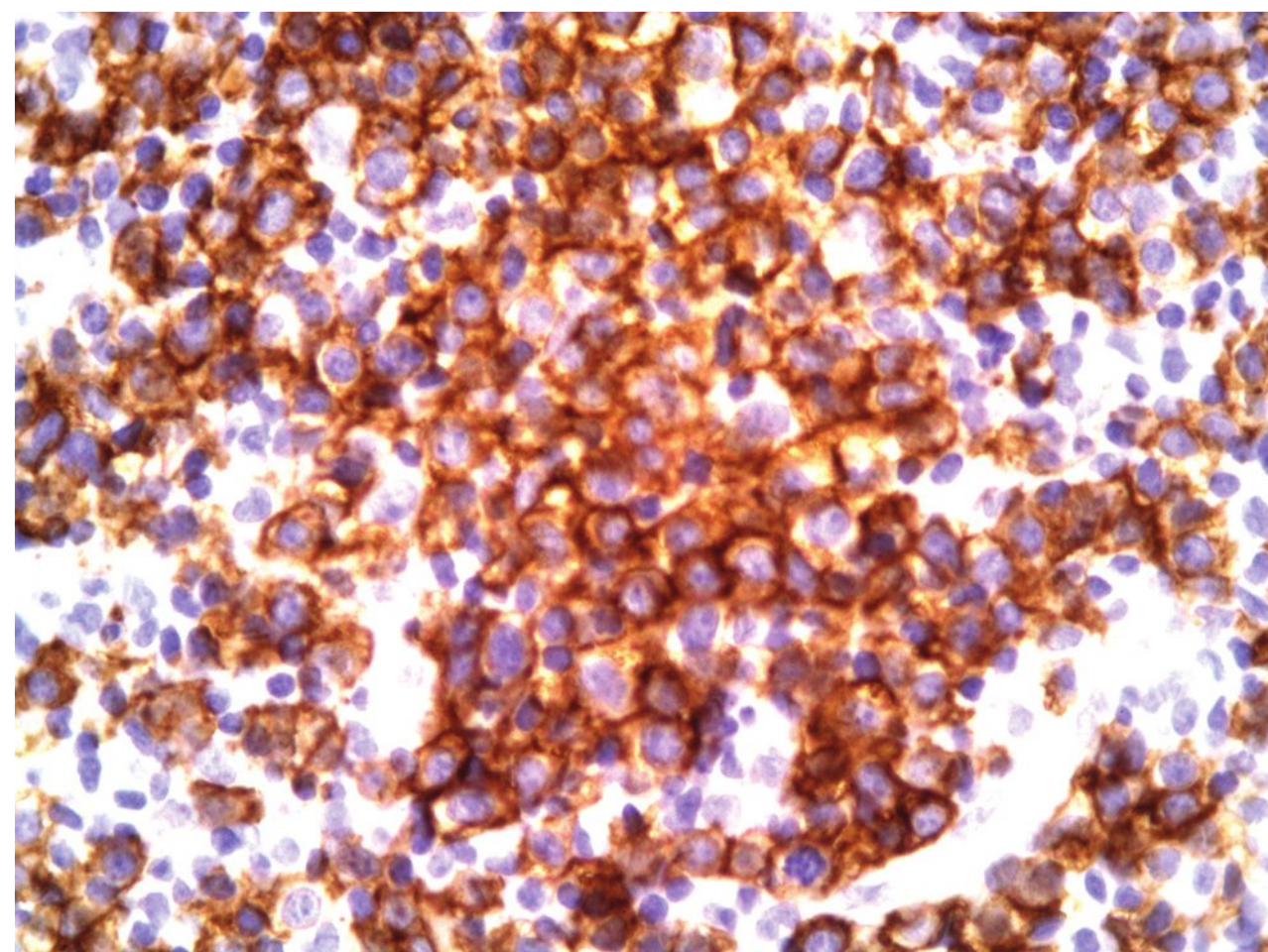


FIGURE 6.32.7 DLBCL in a lymph node biopsy shows CD20 staining. Immunoperoxidase, 60× magnification.

CD10, bcl-2 (Fig. 6.32.8), and bcl-6 are present and make it difficult to distinguish from follicular lymphoma (13–15). In fact, this subset of DLBCL may well be transformed from follicular lymphoma (15). Current studies suggest the division of DLBCL into two subgroups on the basis of the immunophenotype. Cases with CD10 expression in more than 30% of cells or cases with the CD10–, bcl-6+, IRF4/MUM1– phenotype are classified as germinal center–like subgroup. Cases with other immunophenotypes are considered non-germinal center–like subgroup (16). In normal germinal B-cells, the expression of IRF4/MUM1 and bcl-6 is mutually exclusive, but coexpression of both markers was found in 50% of DLBCL cases in one study (4).

CD5 expression has been found in 10% of DLBCL patients, which represent primary DLBCL rather than transformation from small lymphocytic lymphoma/chronic lymphocytic leukemia (17). The absence of cyclin D1

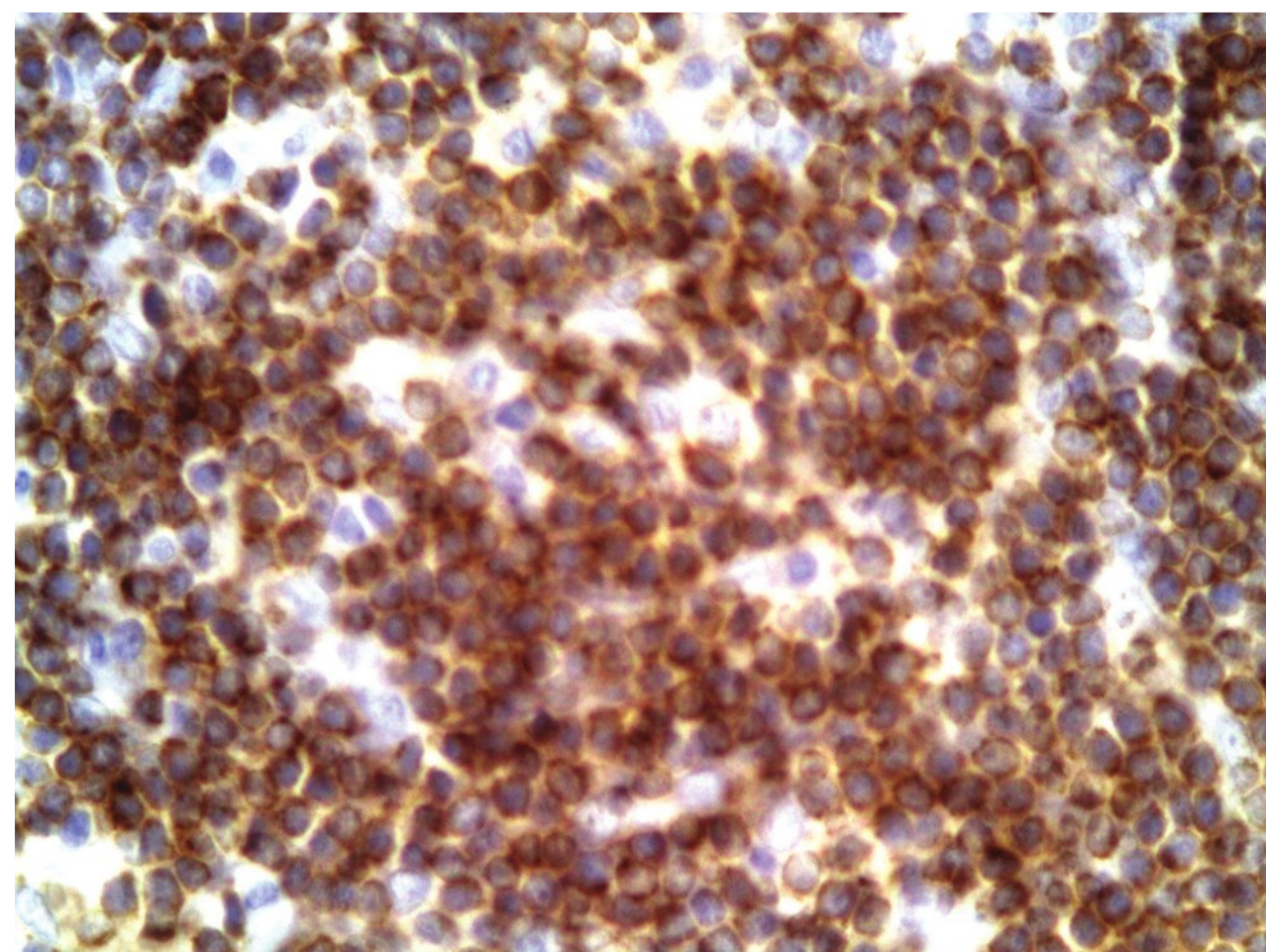


FIGURE 6.32.8 DLBCL in a lymph node biopsy shows bcl-2 staining. Immunoperoxidase, 60× magnification.

expression in these cases distinguishes them from blastoid variants of mantle cell lymphoma (1).

Besides CD5, DLBCL cells do not express T-cell markers, but the infiltrating T cells have great influence on the prognosis. The presence of >20% of infiltrating CD4+ T cells in the pretreatment biopsy is associated with longer relapse-free and overall survival (18), whereas the presence of 15% cytotoxic (CD8+) T cells in the biopsy is associated with a poor progression-free and overall survival time (19).

In the anaplastic variant, CD30 is positive with a membrane or cytoplasmic staining, but CD30 can also be expressed in other variants of DLBCL (20). Other activation antigens, such as CD21, CD23, CD25, CD38, and CD71, can also be shown in this variant (6).

Some cases of DLBCL may show a starry-sky pattern and a high proliferation index mimicking Burkitt lymphoma. If they are the germinal center B-cell-like subgroup, the immunophenotypes of both entities are also similar, except for the absence of bcl-2 expression in Burkitt lymphoma. Recently, it has been found that CD44 and CD45 are also underexpressed in Burkitt lymphoma as compared to DLBCL and can help for differential diagnosis (21).

In plasmablastic lymphoma, the tumor cells are positive for CD38, CD138, Vs38c, and IRF4/MUM1 but are negative or weakly positive for CD45, CD20, and PAX5 (8). The only positive B-cell marker is CD79a, which is expressed in 50% to 85% of the cases (8). Cytoplasmic immunoglobulin is present in 50% to 70% of the cases (8). Ig heavy-chain (IgH) gene rearrangement is demonstrated in all case studied (9). The absence of monoclonal gammopathy distinguishes it from plasmacytoma.

The ALK-positive LBCL is characterized by the presence of strongly positive cytoplasmic ALK protein (11,12). A few cases may show nuclear or nucleolar ALK staining. Otherwise, the immunophenotype mimics myeloma with positive CD38, CD138, and cytoplasmic immunoglobulin (11,12). Lineage-associated markers, such as CD3, CD20, and CD79a, are negative. The absence of CD30 expression helps to exclude anaplastic large-cell lymphoma. This tumor expresses strong epithelial membrane antigen (EMA) and occasional cytokeratin, but weak or negative CD45. These finding may lead to the misdiagnosis of carcinoma.

Comparison between Flow Cytometry and Immunohistochemistry

Flow cytometric analysis usually can identify a monoclonal B-cell population with large cell size so that it substantiates the diagnosis of DLBCL in conjunction with the presence of large cells in tissue sections. Immunohistochemistry is instrumental in the differential diagnosis, such as demonstration of CD30 in the anaplastic variant; detection of VS38c, CD38, and CD138 in plasmablastic lymphoma; and identification of ALK in ALK-positive LBCL.

Molecular Genetics

All variants of DLBCL show somatic mutations in the variable region of the IgH gene; these mutations suggest that the DLBCL cells are derived from germinal center or postgerminal center B cells (1,6). However, IgH gene rearrangement was not demonstrated with the PCR

technique in 30% to 50% of DLBCL cases in one study; the authors considered that the high number of somatic mutations might inhibit primer annealing (6). One study found that patients with IgH gene rearrangement detected in the blood or bone marrow had a lower complete remission rate and a significantly poorer overall survival than the patients with negative results (22).

Using DNA microarrays, two gene expression profiles were demonstrated by Alizadeh et al. (23): the germinal center B-cell-like subgroup and the activated B-cell-like subgroup. The former was associated with a good outcome and the latter with a poor outcome. A subsequent study by Rosenwald et al. (24) identified a third group, which did not express either set of genes from the above two groups. This study suggested that the difference in the prognosis is associated with the activity of the nuclear factor- κ B signaling pathway. This pathway interferes with the apoptotic effect of chemotherapy. The germinal center B-cell-like subgroup inhibits and the activated B-cell-like subgroup enhances this pathway; thus, the latter subgroup could block the apoptosis induced by chemotherapy, leading to a poor outcome (24).

Rosenwald et al. (24) also identified t(14;18) translocation and amplification of the c-REL locus exclusively in the germinal center B-cell-like subgroup, indicating that it is a distinct disease entity. By using selective “predictive” genes, these authors further stratified four biologic groups with distinctly different prognoses.

The morphologic variants distribute variably in different gene expression profiling subgroups (24). The centroblastic monomorphic variants are seen mainly in the germinal center B-cell-like subgroup, whereas the centroblastic polymorphic and immunoblastic variants are more common in the activated B-cell-like subgroup.

The gene expression profiling subgroups are associated with different immunophenotypes (25,26). The germinal center B-cell-like subgroup expresses bcl-6+/CD10±/MUM1-/CD138- or bcl-6-/CD10+/MUM1-/CD138-. The non-germinal center B-cell-like subgroup shows bcl-6+/CD10-/MUM1+/CD138-. In another study, the activated B-cell phenotype, as defined by CD20+, cIgM+, MUM-1+, CD138±, bcl-6-, was found to be associated with the plasmablastic variant (27). This group of patients had a high frequency (85%) of tumor suppressor protein p53 (TP53) deletions, leading to a poor response to chemotherapy and short survival.

In terms of oncogenes, BCL-6 is the most common cytogenetic defect in DLBCL (6). It was initially considered specific for this lymphoma, but it was later found that this anomaly can also be demonstrated in a significant number of follicular lymphomas. Bcl-6 may be translocated with IgH, with a resultant karyotype of t(3;14)(q27;q32) or with other partner genes, such as t(3;6)(q29;p15) or t(3;22)(q27;q11) (5). BCL-6 is required for formation of germinal centers and its downregulation is required for B-cells to undergo further differentiation to memory cells or plasma cells (28). However, BCL-6 also attenuates DNA damage sensing by mediating transcriptional repression of ataxia telangiectasia and Rad3 related (ATR). Through the actions of BCL-6, centroblasts fail to trigger an ATR response for DNA damage repair, which probably plays a fundamental role in lymphomagenesis in DLBCL (28).

Another oncogene, BCL-2, is also commonly present in DLBCL with a frequency of 20% to 30% in the form of t(14;18) (q32;q21) (29). BCL-1 aberration is absent in DLBCL. MYC expression is generally considered uncommon, but, with the real-time reverse transcription (RT)-PCR technique, 30% of DLBCL cases showed overexpression of MYC (30). MYC rearrangement has been observed in up to 10% of DLBCL cases (4). The partner gene is an Ig gene in 60% and a non-Ig gene in 40% of cases (4). Cases with concurrent BCL-2 and/or BCL-6 translocation are better categorized as B-cell lymphoma, unclassified, with features intermediate between DLBCL and Burkitt lymphoma. For numerical aberrations, the most frequent changes are gains of X, 3q, 7, 12q, and 18q and loss of 6q and 17p (31).

The current case is unusual in that the patient initially presented with splenomegaly. The morphologic diagnosis was anaplastic variant of DLBCL. The immunophenotype by flow cytometry was that of a B-cell lymphoma with negative CD5 and CD10, whereas immunohistochemical study revealed the characteristic CD30 marker for the anaplastic variant. The patient was treated successfully for 5 years until he had recurrent lymphoma discovered in the lymph node. The complex cytogenetic karyotype identified in the lymph node was suggestive of a high-grade lymphoma. The patient died rapidly after the recurrence. The salient features for laboratory diagnosis of DLBCL are summarized in Table 6.32.3.

Clinical Manifestations

DLBCL is commonly seen in old people with a median age in the seventh decade, but it may be present (with a low frequency) in children (1). The characteristic clinical presentation is a rapidly growing symptomatic mass, mostly in the neck or abdomen due to lymphadenopathy (32). Systemic B symptoms, such as fever, night sweats, and weight loss of >10%, are seen in one quarter of patients,

and elevated serum lactate dehydrogenase is present in one third of patients. Approximately 20% of patients have stage I or stage IE disease (33), whereas 40% of patients present with stage IV disease at diagnosis (34,35).

Extranodal dissemination of DLBCL can be seen in 40% of patients (34). The sites involved include gastrointestinal tract, genitourinary system, central nervous system, skin, liver, paranasal sinuses, endocrine glands, and others. Bone marrow involvement occurs in 10% to 20% of cases and is usually associated with later spread to the central nervous system (35,36).

Although there are sporadic reports of poor prognosis for some morphologic subtypes, the prognosis of DLBCL is generally not correlated with morphology. However, several studies show that the clinical outcomes are associated with the degree and pattern of bone marrow infiltration as well as the presence of concordant or discordant involvement (37). An extensive, diffuse infiltration pattern with large cells (concordant) usually predicts a poor prognosis.

The international prognostic index including age, Eastern Cooperative Oncology Group performance status, tumor stage, lactate dehydrogenase level, and the number of extranodal sites involved has proved to be valuable to predict the prognosis of DLBCL patients (24). The expression of CD138 (26) and a high proliferative index demonstrated by Ki-67 staining (1) are also predictors for a poor outcome. However, molecular genetic markers are most reliable. For instance, the BCL-2 expression and p53 overexpression are associated with poor prognosis, whereas BCL-6 translocation is associated with a favorable prognosis (1). The most powerful tool is the gene expression profiling, which stratifies patients into prognostic groups (23,24). A recent study pinpoints six genes in the gene expression profile that correlate well with the prognosis of DLBCL (38). The combination of LMO2, BCL-6, and FN1 is associated with a better prognosis, whereas that of BCL-2, SCYA3, and CCND2 is associated with worse prognosis.

The pediatric case of DLBCL differs from that of adult cases in its morphologic, immunologic, and genetic homogeneity (39). For instance, pediatric cases are more frequently of the monomorphic centroblastic variant, predominantly of germinal center B-cell-like subtype, and often expressing CD10 and bcl-6. Therefore, the treatment of pediatric cases should be specifically tailored and not necessarily follow the adult therapeutic protocol (39).

TABLE 6.32.3

Salient Features of Laboratory Diagnosis of DLBCL

1. General expression of B-cell antigens: CD19, CD20, CD22, CD79a, and monoclonal surface immunoglobulin
2. The germinal center B-cell-like subgroup shows CD10+, bcl-6-, IRF4/MUM1- or CD10-, bcl-6+, IRF4/MUM1-.
3. The non-germinal center B-cell-like subgroup shows CD10-, bcl-6±, IRF4/MUM1+
4. A CD5-positive subgroup is seen in the minority of cases.
5. Anaplastic variant or occasionally other variants may express CD30.
6. Monoclonal immunoglobulin heavy-chain gene rearrangement
7. BCL-2 or BCL-6 gene rearrangement is present in certain subsets.

REFERENCES

1. Gatter KC, Warnke RA. Diffuse large B-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:171–174.
2. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas. *Cancer*. 1982;49:2112–2135.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.

4. Stein H, Warnke RA, Chan WC, et al. Diffuse large B-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:233–237.
5. Morton LM, Wang SS, Devesa SS, et al. Lymphoma incidence patterns by WHO subtype in the United States, 1992–2001. *Blood*. 2006;107:265–276.
6. Anagnostopoulos I, Dallenbach F, Stein H. Diffuse large cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:855–914.
7. Lennert K, Feller A. *Histopathology of Non-Hodgkin's Lymphomas (Based on the Updated Kiel Classification)*. 2nd ed. New York: Springer-Verlag; 1990.
8. Stein H, Harris NL, Campo E. Plasmablastic lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:256–257.
9. Delecluse HJ, Anagnostopoulos I, Dallenbach F, et al. Plasmablastic lymphomas of the oral cavity: a new entity associated with the human immunodeficiency virus infection. *Blood*. 1997;89:1413–1420.
10. Brown RS, Campbell C, Lishman SC, et al. Plasmablastic lymphoma: a new subcategory of human immunodeficiency virus-related non-Hodgkin's lymphoma. *Clin Oncol*. 1998;10:327–329.
11. Delsol G, Campo E, Gascoyne RD. ALK-positive large B-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC Press; 2008:254–255.
12. Delsol G, Lamant L, Mariame B, et al. A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2:5 translocation. *Blood*. 1997;89:1483–1490.
13. Frost M, Newell J, Lones MA, et al. Comparative immunohistochemical analysis of pediatric Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Clin Pathol*. 2004;121:384–392.
14. Xu Y, McKenna RW, Molberg KH, et al. Clinicopathologic analysis of CD10+ and CD10– diffuse large B-cell lymphoma: identification of a high-risk subset with coexpression of CD10 and bcl-2. *Am J Clin Pathol*. 2001;116:183–190.
15. Bertram HC, Check IJ, Milano MA. Immunophenotyping large B-cell lymphomas: flow cytometric pitfalls and pathologic correlation. *Am J Clin Pathol*. 2001;116:191–203.
16. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103:275–282.
17. Matolcsy A, Chadburn A, Knowles DM. De novo CD5-positive and Richter's syndrome-associated diffuse large B cell lymphomas are genotypically distinct. *Am J Pathol*. 1995;147:207–216.
18. Ansell SM, Stenson M, Habermann TM, et al. CD4+ T-cell immune response to large B-cell non-Hodgkin's lymphoma predicts patient outcome. *J Clin Oncol*. 2001;19:720–726.
19. Muris JJ, Meijer CJ, Cillessen SA, et al. Prognostic significance of activated cytotoxic T-lymphocytes in primary nodal diffuse large B-cell lymphomas. *Leukemia*. 2004;18:589–596.
20. Piris M, Brown DC, Gatter KC, et al. CD30 expression in non-Hodgkin's lymphoma. *Histopathology*. 1990;17:211–218.
21. Schniederjan SD, Li S, Saxe DF, et al. A novel flow cytometric antibody panel for distinguishing Burkitt lymphoma from CD10+ diffuse large B-cell lymphoma. *Am J Clin Pathol*. 2010;133:718–726.
22. Mitterbauer-Hohendanner G, Mannhalter C, Winkler K, et al. Prognostic significance of molecular staging by PCR-amplification of immunoglobulin gene rearrangements in diffuse large B-cell lymphoma (DLBCL). *Leukemia*. 2004;18:1102–1107.
23. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403:503–511.
24. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346:1937–1948.
25. Bai M, Skyras A, Agnantis NJ, et al. Diffuse large B-cell lymphomas with germinal center B-cell-like differentiation immunophenotypic profile are associated with high apoptotic index, high expression of the proapoptotic proteins bax, bak and bid and low expression of the antiapoptotic protein bcl-xl. *Mod Pathol*. 2004;17:847–856.
26. Oh YH, Park CK. Prognostic evaluation of nodal diffuse large B cell lymphoma by immunohistochemical profiles with emphasis on CD138 expression as a poor prognostic factor. *J Korean Med Sci*. 2006;21:397–405.
27. Simonitsch-Klupp I, Hauser I, Ott G, et al. Diffuse large B-cell lymphomas with plasmablastic/plasmacytoid features are associated with TP53 deletions and poor clinical outcome. *Leukemia*. 2004;18:146–155.
28. Ci W, Polo JM, Melnick A. B-cell lymphoma 6 and the molecular pathogenesis of diffuse large B-cell lymphoma. *Curr Opin Hematol*. 2008;15:381–390.
29. Weiss LM, Warnke RA, Sklar J, et al. Molecular analysis of the t(14;18) chromosomal translocation in malignant lymphomas. *N Engl J Med*. 1987;317:1185–1189.
30. Saez AI, Artiga MJ, Romero C, et al. Development of a real-time reverse transcription polymerase chain reaction assay for c-myc expression that allows the identification of a subset of c-myc+ diffuse large B-cell lymphoma. *Lab Invest*. 2003;83:143–152.
31. Berglund M, Enblad G, Flordal E. Chromosomal imbalances in diffuse large B-cell lymphoma detected by comparative genomic hybridization. *Mod Pathol*. 2002;15:807–817.
32. Moller MB, Pedersen NT, Christensen BE. Diffuse large B-cell lymphoma: clinical implications of extranodal versus nodal presentation—a population-based study of 1575 cases. *Br J Haematol*. 2004;124:151–159.
33. Grosskreutz C, Troy K, Cuttner J. Primary splenic lymphoma: report of 10 cases using the REAL classification. *Cancer Invest*. 2002;20:749–753.
34. Paryani S, Hoppe R, Burke J, et al. Extranodal involvement in diffuse non-Hodgkin's lymphoma. *J Clin Oncol*. 1983;1:682–688.
35. Rudders R, Ross M, Delellis R. Primary extranodal lymphoma. *Cancer*. 1978;42:406–416.
36. van Besien K, Ha C, Murphy S, et al. Risk factors, treatment, and outcome of central nervous system recurrence in adults with intermediate-grade and immunoblastic lymphoma. *Blood*. 1998;91:1178–1184.
37. Talaulikar D, Dahlstrom JE. Staging bone marrow in diffuse large B-cell lymphoma: the role of ancillary investigations. *Pathology*. 2009;41:214–222.
38. Lossos IS, Czerwinski DK, Alizadeh AA, et al. Prediction of survival in diffuse large-B-cell lymphoma based on the expression of six genes. *N Engl J Med*. 2004;350:1828–1837.
39. Reiter A, Klapper W. Recent advances in the understanding and management of diffuse large B-cell lymphoma in children. *Br J Haematol*. 2008;142:329–347.

CASE 33

T-Cell/Histiocyte-Rich Large B-Cell Lymphoma

CASE HISTORY

A 70-year-old man presented with an approximately 1.5-week history of chest pain. The chest pain radiated to both shoulders and arms and was associated with breathing difficulty. Physical examination revealed no lymphadenopathy and hepatosplenomegaly. Peripheral blood examination showed a total leukocyte count of 14,500/mL with 77.5% neutrophils, 12.2% lymphocytes, 9.1% monocytes, 1.2% eosinophils, and 0.2% basophils. His hemoglobin was 15.7 g/dL, hematocrit 45.4%, mean cell volume 95.1 fL, mean cell hemoglobin 33.0 pg, and platelets 279,000/mL. His blood chemistry panel was unremarkable except for an elevated level of lactate dehydrogenase (282 IU/L). While in the emergency department, the patient was found to have a right hilar fullness on chest x-ray. Subsequent CT scan revealed a large 11 × 5 cm mass in the posterior mediastinal space with compression of subclavian vein.

After admission, an endobronchial biopsy of the right lower lobe of the lung showed no diagnostic abnormality. A mediastinal biopsy finally demonstrated a malignant lymphoma (Fig. 6.33.1). Subsequently, the patient received five cycles of elective chemotherapy. The patient responded well to chemotherapy at the beginning, but he had multiple medical problems, including hypertension, aortic aneurysm, acute renal insufficiency, and fungemia. He died 15 months after the initial diagnosis.

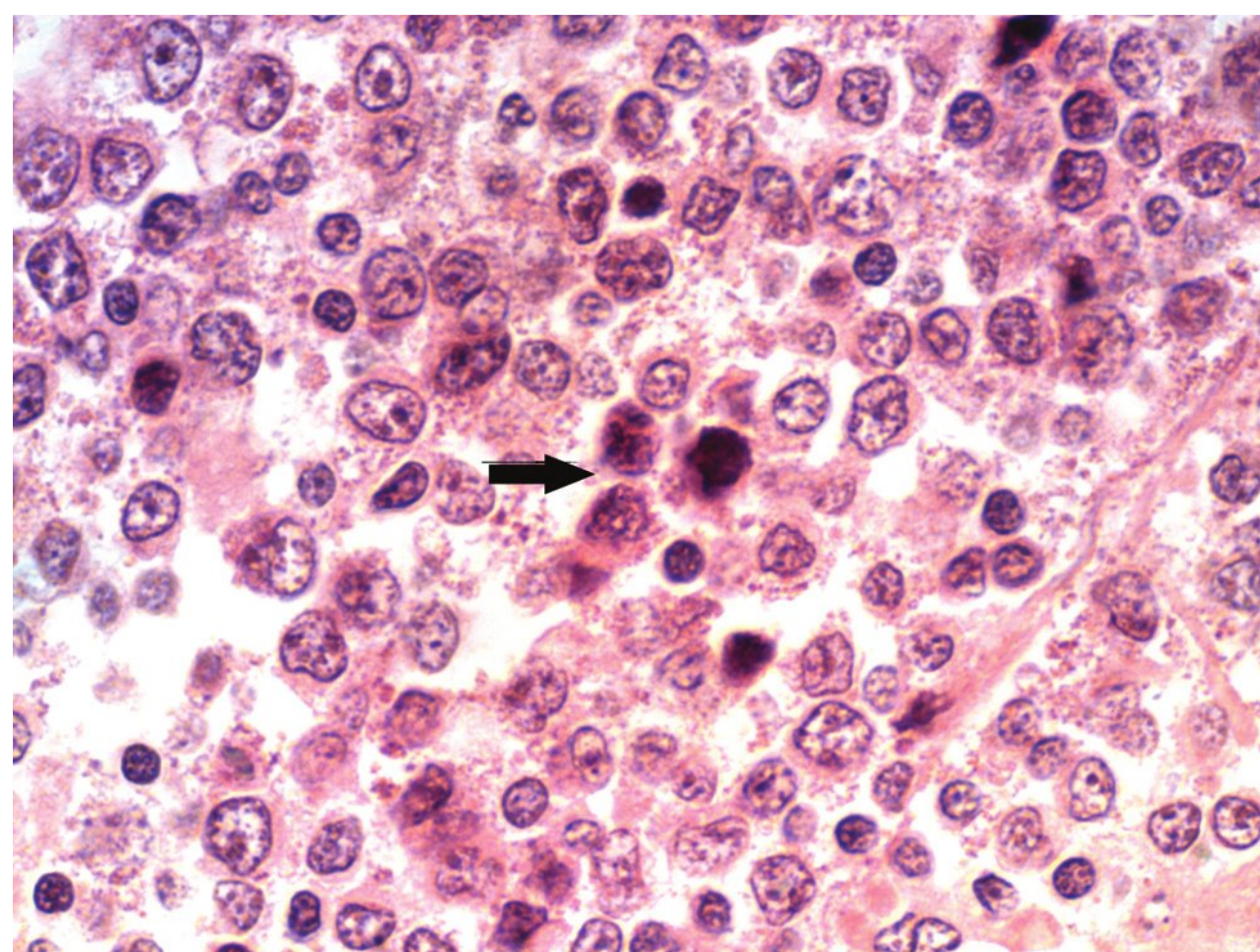


FIGURE 6.33.1 Lymph node biopsy shows three hyperchromatic tumor cells (arrow) amid a background of histiocytes (larger cells) and lymphocytes (small cells). H&E, ×100.

IMMUNOHISTOCHEMISTRY

Immunohistochemical stains of the mediastinal lymph node showed that a few scattered large tumor cells were positive for CD20 (Fig. 6.33.2) but were negative for CD15 and CD30. CD3 (Fig. 6.33.3) and CD68 (Fig. 6.33.4) stains demonstrated numerous small lymphocytes and medium-sized histiocytes, respectively. No follicular dendritic cell meshwork was identified by CD21 stain.

DISCUSSION

T-cell/histiocyte-rich large B-cell lymphoma (THRBCL) is a subtype of diffuse large B-cell lymphoma and is characterized by the presence of fewer than 10% of large tumor cells of B-cell origin on a background of large numbers of T lymphocytes and histiocytes. THRBCL was called pseudo-T-cell lymphoma in the early literature (1). Ramsay et al. (2) coined the term T-cell-rich B-cell lymphoma in 1988 and defined this lymphoma as a B-cell lymphoma with more than 90% of reactive T cells. Several subsequent reports used this term loosely to include B-cell lymphoma with a large T-cell population, between 30% and 90% (3–6). In 1992, Delabie et al. (7) introduced a new term “histiocyte-rich B-cell lymphoma” to include B-cell lymphoma cases with high numbers of histiocytes and small lymphocytes. However, as these cases invariably contained many T cells, this new term was seldom used (8,9). The current term THRBCL was adopted by the World Health Organization (WHO) classification in 2001 and was categorized as a morphologic variant of diffuse

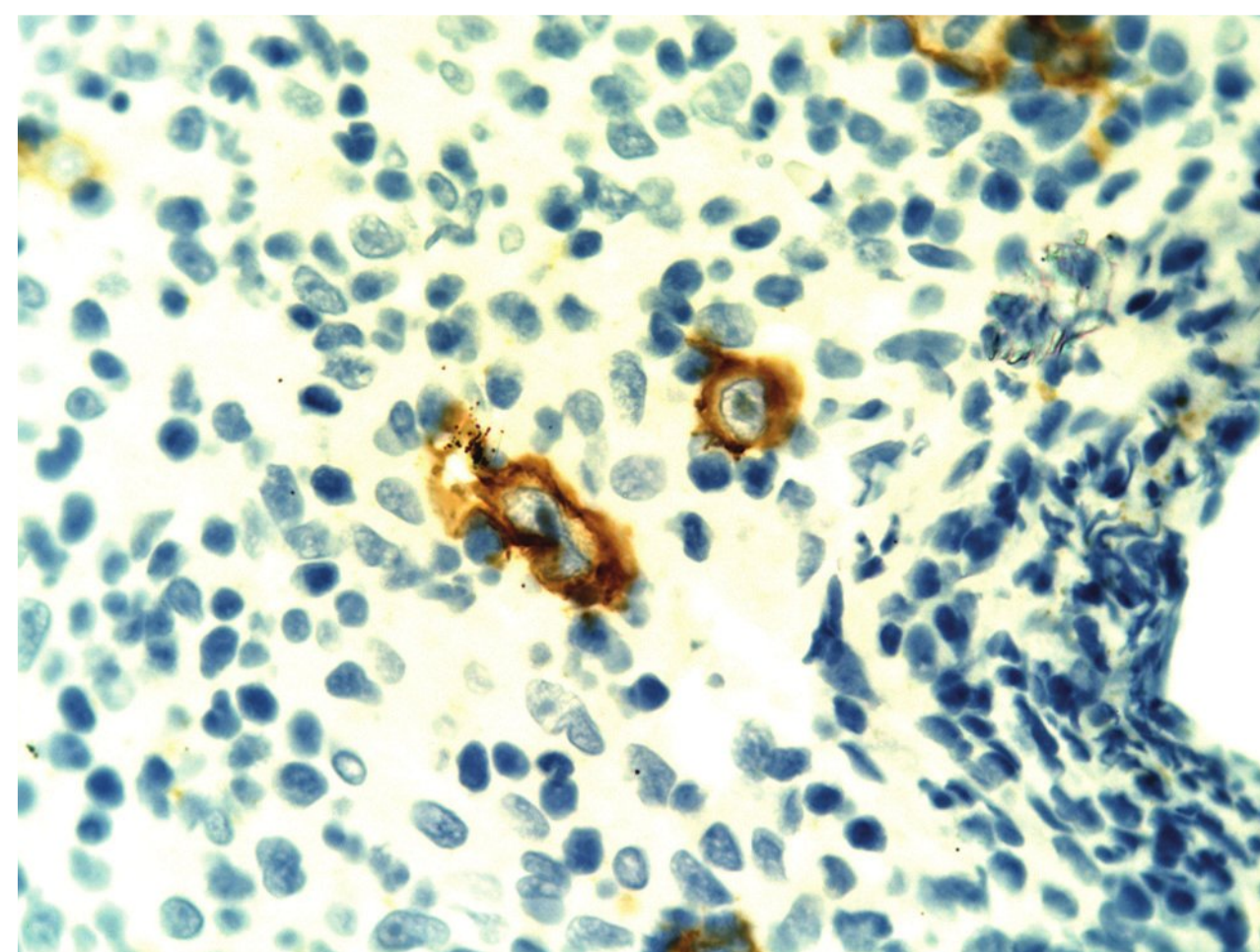


FIGURE 6.33.2 CD20 stain demonstrates a few large tumor cells. Immunoperoxidase, ×60.

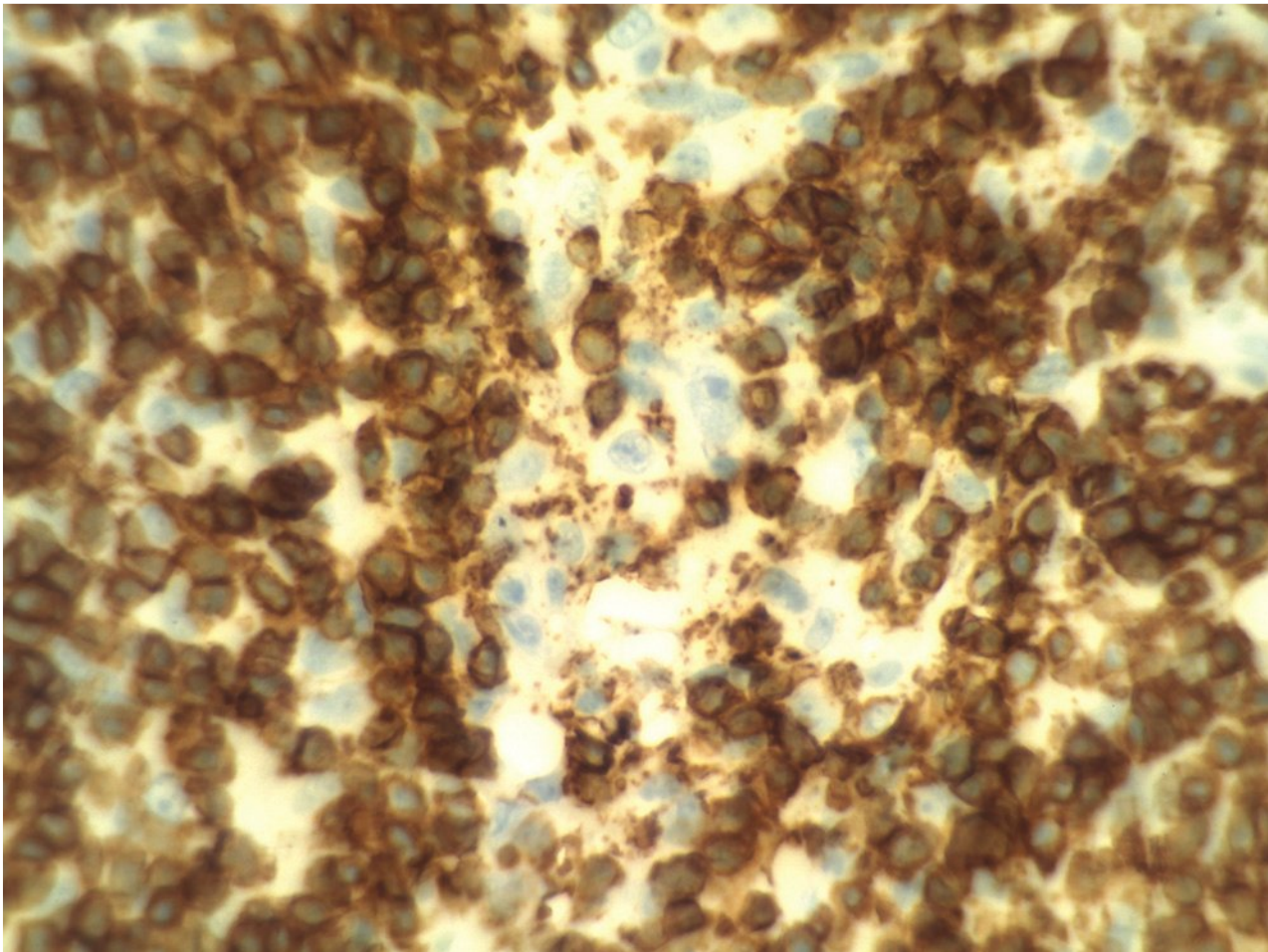


FIGURE 6.33.3 CD3 stain shows many T lymphocytes. The unstained cells represent histiocytes and tumor cells. Immunoperoxidase, $\times 40$.

large B-cell lymphoma (10). In the 2008 WHO classification, THRBCL is categorized as a distinct pathologic entity (11).

Morphology

THRBCL is characterized by the effacement of the normal lymph node architecture by extensive histiocyte and small lymphocyte infiltration with scattered large tumor cells that are fewer than 10% of the total population (11–17). In a minority of cases, a vaguely nodular pattern may be discernable. In some cases, the tumor cells may be so inconspicuous that immunohistochemistry is required for their recognition. The morphology of tumor cells differs from case to case; they may be manifested as centroblasts, immunoblasts, Reed-Sternberg–like cells, or lymphocyte predominant cells (LP cells or popcorn cells) or the combination of two or more cell types. The background cells, though not neoplastic, are important in the differential

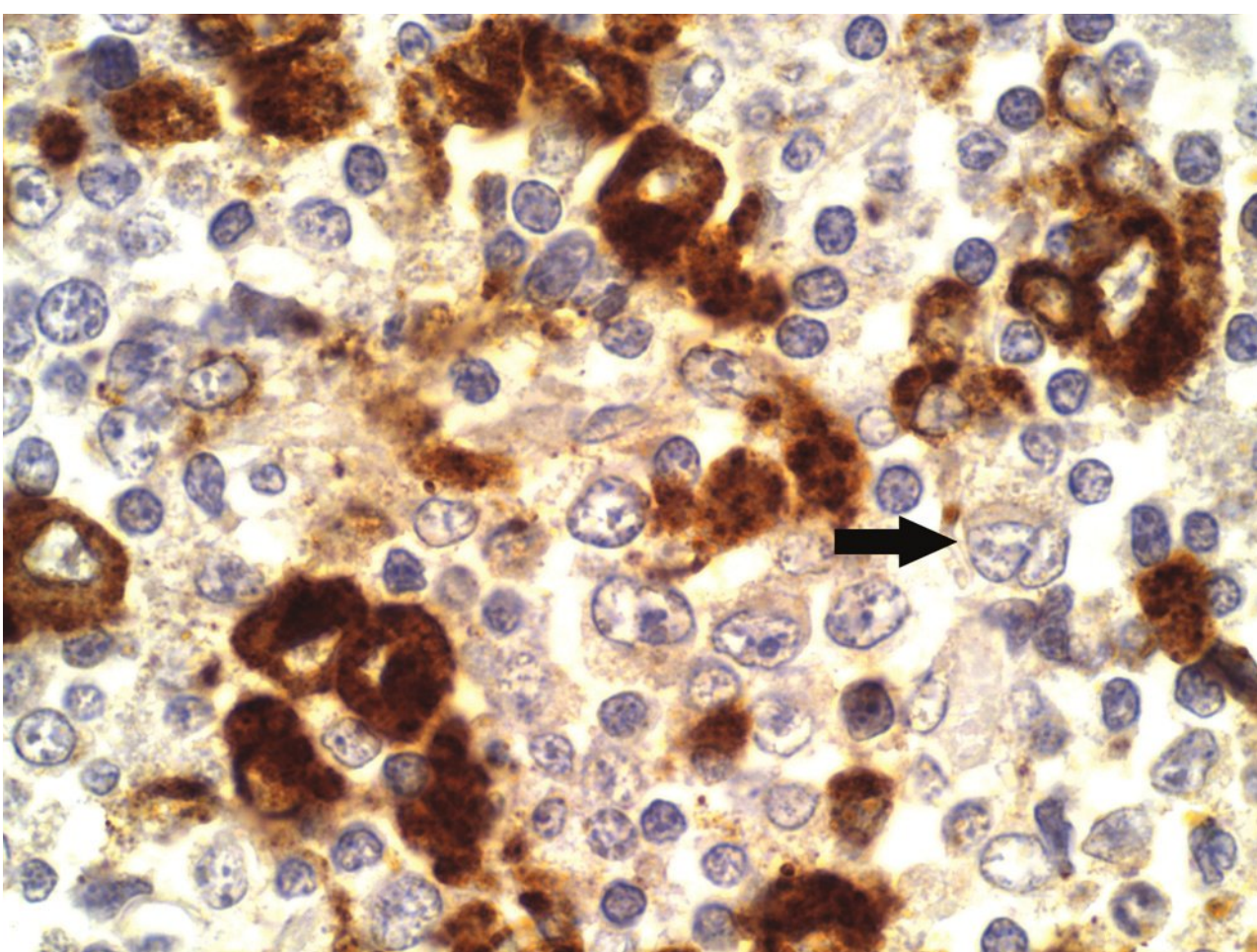


FIGURE 6.33.4 CD68 stain identifies many atypical histiocytes. There are a few unstained tumor cells (arrow) and numerous small lymphocytes. Immunoperoxidase, $\times 100$.

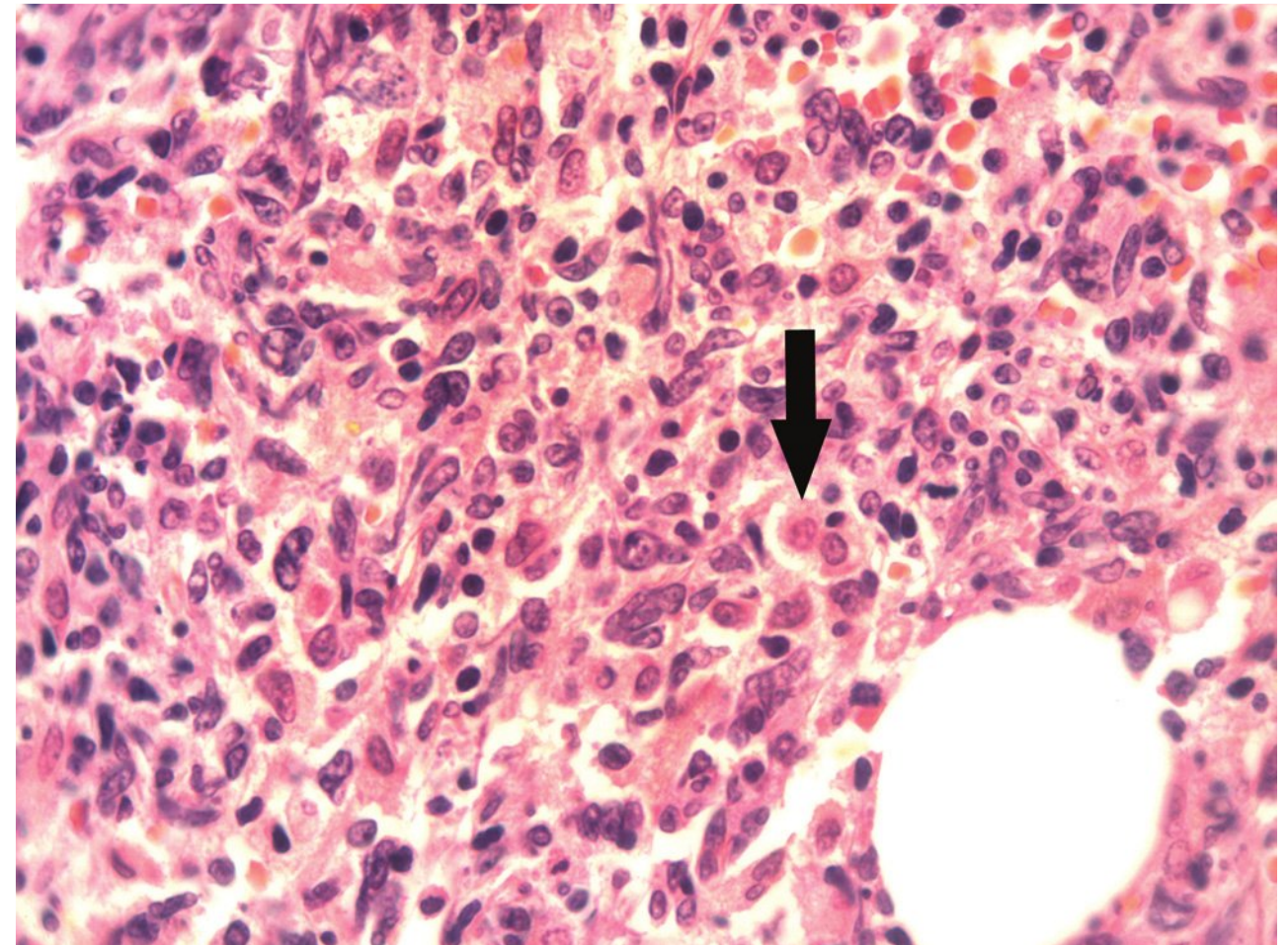


FIGURE 6.33.5 Bone marrow biopsy reveals many histiocytes and small lymphocytes with a few scattered tumor cells (arrow). H&E, $\times 60$.

diagnosis by their immunophenotype and are also a major contributing component in the gene expression profiling of THRBCL. The lack of eosinophils, neutrophils, and plasma cells helps distinguish THRBCL from Hodgkin lymphomas.

THRBCL is also characterized by its frequent extranodal distribution, particularly the spleen, liver, and bone marrow (Fig. 6.33.5) (12,18). A few cases of primary splenic THRBCL, which are characterized by a micronodular pattern, have been reported (19–22). The tumor nodules are composed of histiocytes and small lymphocytes with rare large tumor cells. The bone marrow also shows a nodular pattern with the same composition as that in the spleen (15,19). In the liver, the lymphomatous foci are localized in the portal tracts (19).

THRBCL is very similar to nodular lymphocytic predominant Hodgkin lymphoma (NLPHL) morphologically and immunologically. The tumor cells of both entities have nearly identical immunophenotype. The prominent nodular pattern and the cellular milieu in NLPHL may help to distinguish it from THRBCL, but it is the immunophenotype of the background cells that can provide a definitive distinction. In several studies, there was a minority of cases that showed overlapped features between THRBCL and NLPHL in both morphology and immunophenotype (13,23). These cases are designated “gray zone” lymphoma. In addition, there is morphologic documentation that THRBCL can be coexistent with or subsequent to NLPHL in the clinical course (23–26). Some authors believe that these cases represent transformation from NLPHL to THRBCL (11,13,26).

THRBCL may also mimic classical Hodgkin lymphoma (cHL) for its scarcity of tumor cells and an inflammatory background. As mentioned before, THRBCL may also show Reed-Sternberg–like cells. However, cHL also contains other tumor cells, such as lacunar cells, mononucleated and multinucleated Hodgkin cells as well as mummified cells. Eosinophils, neutrophils, and plasma cells are also seen in cHL and not in THRBCL. Finally, the immunophenotype of both tumor cells and background cells is quite different between these two entities.

When the rare tumor cells are missed or not demonstrated by immunophenotyping, THRBCL may be misdiagnosed as inflammatory reaction or peripheral T-cell lymphoma. Indeed, THRBCL is frequently mistaken as other lymphomas or reactive conditions by the primary pathologists; one study showed that the initial diagnoses were incorrect in as high as 82% of the cases studied (18).

Immunophenotype

Immunophenotyping is the mainstay for the diagnosis of THRBCL (11–17,27). The tumor cells can be readily identified by immunohistochemical staining of CD20, but they are also consistently positive for the B-cell transcription factors, including PAX5/BSAP, OCT2, and BOB1 (11–17,27). One transcription factor, PU.1 is usually negative in THRBCL cases and is thus useful for distinguishing THRBCL from NLPHL, which is positive for this marker. The major markers for differentiating cHL from non-Hodgkin lymphoma are CD15, CD30, and CD45. While most non-Hodgkin lymphomas are CD45 positive but CD15 and CD30 negative, the tumor cells in THRBCL can be weakly positive for CD30. CD79a and BCL6 are demonstrated in most cases, while CD10 is only present in the minority of cases. BCL-2 expression is variable (14% to 50%) in different study series (12). Surface immunoglobulin, J-chain, and epithelial membrane antigen (EMA) are seldom used for the diagnosis, but they have been reported to be present in a minority of cases (12–14,17). CD138 stain has been performed in a few studies and is always negative (15,16,27,28). Epstein–Barr virus (EBV) is seldom demonstrated by latent membrane protein-1 (LMP-1) staining or EBV-encoded RNA hybridization (EBER) technique (14,17,29,30). The WHO classification states that if a T-cell-rich B-cell lymphoma shows positive EBV stains, such cases should be considered within the spectrum of EBV positive diffuse large B-cell lymphoma (11).

Unlike other lymphomas, immunophenotyping of the nonneoplastic background cells is most important for the differential diagnosis of THRBCL. The histiocytes in this entity can be highly pleomorphic and should be distinguished from the tumor cells by CD68 staining. The T lymphocytes are CD3 positive, similar to the background cells in Hodgkin lymphoma, but unlike the latter, these CD3 positive cells do not form rosettes around the tumor cells. The T cells are also CD57 negative in THRBCL cases. Some studies reported that the T cells in THRBCL are CD8-positive cytotoxic T cells, so that they express cytotoxic proteins (13), but this property has not been characterized in most studies. Another important marker for the differential diagnosis is CD21, which is able to demonstrate the follicular dendritic cell meshwork in classical and non-classical Hodgkin lymphoma but not in THRBCL. Another characteristic feature of the cellular milieu is the rarity of B lymphocytes in THRBCL so that CD20 staining should be negative for most of the small lymphocytes.

On the basis of the immunophenotype (CD20+, CD79a+, BCL-6+, CD138–), most authors consider THRBCL as derived from the germinal center B cells (11,13–15). The absence of CD138 is not consistent with the postgerminal center phenotype. The absence of CD10 and BCL-2 in many cases does not necessarily negate the germinal center origin, as BCL-6 is the most reliable marker for germinal

center B cells. BCL-6 protein is a POZ/zinc finger transcriptional repressor that is expressed selectively by germinal center B cells but not by immature B-cell precursors or plasma cells (14). Some authors even assert that cases of THRBCL that fail to express BCL-6 could still be descended from a germinal center ancestor but in the course of malignant transformation, BCL-6 might have lost (15). There was only one CD5-positive THRBCL case reported, in which the tumor cells expressed CD5, CD19, BCL-6, and MUM1/IRF4 but not CD138 (28). The authors considered this case representing a tumor derived from late germinal center or early postgerminal center B cells.

The tumor cells in NLPHL are positive for CD45, CD20, OCT2, BOB1, PU.1, BCL-6 CD79a, and EMA, but are negative for the traditional Hodgkin's markers, CD15, and CD30. The background cells in NLPHL are CD3/CD57-positive T cells, forming rosettes with the tumor cells, and polyclonal B cells. A follicular dendritic cell meshwork can be demonstrated by CD21 staining.

The tumor cells in cHL are positive for CD15, CD30, and PAX5/BSAP, but are negative for CD45, CD20, CD79a, BOB1, OCT2, and PU.1. The background cells are mainly CD3 T cells with rare CD20 B cells, except for the lymphocyte-rich subtype. CD38-positive histiocytes can be present in some subtypes and the number is variable. A follicular dendritic cell meshwork is present. The differences between THRBCL, NLPHL, and cHL are listed in Table 6.33.1 (12,13,17).

Comparison of Flow Cytometry and Immunohistochemistry

As there are so few tumor cells present, flow cytometry plays no role in the diagnosis of THRBCL. A monoclonal B-cell population is seldom identified by this technique. Immunohistochemical staining, on the other hand, not only can characterize the tumor cells but also the background cells. For practical purposes, only CD15, CD30, CD45, CD20, and CD3 are needed to characterize the tumor cells. CD3, CD20, CD57, and CD68 are useful for characterization of the background cell composition. CD21 should be used to identify the follicular dendritic cell meshwork.

Molecular Genetics

Immunoglobulin heavy-chain gene rearrangement of THRBCL cases has demonstrated clonal gene rearrangement carrying high numbers of somatic mutations and intraclonal diversity, characteristic of germinal center cells (31). In spite of the frequent expression of BCL-6 protein, BCL-6 gene rearrangement is not seen in THRBCL cases (30). BCL-1 and MYC rearrangements are also not demonstrated in a limited number of studies (30). Clonal BCL-2 gene rearrangement is shown only in rare cases, and this finding is used to argue against the assumption that THRBCL is transformed from follicular lymphoma (14,17).

However, there are morphologic and molecular evidence suggesting that THRBCL can be transformed from NLPHL (23–26). Immunoglobulin heavy-chain gene analysis of the specimens from a patient with both tumors showed that they shared the same complementarity-determining region 3 (32). Comparative genomic hybridization shows that THRBCL and NLPHL share some genomic imbalances, but they also

TABLE 6.33.1			
Differences between THRBCL, NLPHL, and cHL			
	THRBCL	NLPHL	cHL
Clinical course	Aggressive	Indolent	Indolent
Histologic pattern	Diffuse*	Nodular	Nodular or diffuse
Tumor cell morphology	Immunoblast, centroblast, LP cell, or RS cell	LP cell	RS cell
Tumor cell phenotype			
CD45	Positive	Positive	Negative
CD20	Positive	Positive	Negative
CD79a	Positive	Positive	Negative
BCL-6	Positive	Positive	Negative
PAX5/BSAP	Positive	Positive	Positive
BOB1	Positive	Positive	Negative
OCT2	Positive	Positive	Negative
PU.1	Negative	Positive	Negative
CD10	Variable	Negative	Negative
BCL-2	Variable	Negative	Negative
CD15	Negative	Negative	Positive
CD30	Rare	Negative	Positive
EBV	Negative	Negative	Variable
EMA	Variable	Positive	Rare
J-chain	Positive	Positive	Negative
sIg	Positive	Positive	Negative
Background cell phenotype			
CD3	Positive	Positive	Positive
CD57	Negative	Positive	Positive
CD20	Negative	Positive	Variable
CD21	Negative	Positive	Positive
CD68	Positive	Rare	Variable
PD1	Negative	Positive	Negative
IgH gene rearrangement	Monoclonal	Polyclonal	Polyclonal

*Vaguely nodular pattern can be seen in a minority of THRBCL cases and micronodular pattern can be seen in primary splenic THRBCL cases.
cHL, classical Hodgkin lymphoma; EBV, Epstein–Barr virus; EMA, epithelial membrane antigen; IgH, immunoglobulin heavy chain; LP cell, lymphocyte predominant cell (popcorn cell); NLPHL, nodular lymphocyte predominant Hodgkin lymphoma; RS cell, Reed–Sternberg cell; sIg, surface immunoglobulin; THRBCL, T-cell/histiocyte-rich B-cell lymphoma.

have distinctive abnormalities to distinguish from each other (23,33). Franke et al. (33) concluded that THRBCL and NLPHL are two distinct entities, but they may originate from the same precursor cells. Gene expression profiling analysis of diffuse large B-cell lymphoma cases has found that all THRBCL cases belong to the “host response” subgroup, which shows a signature defined primarily by the reactive host inflammatory cells rather than the tumor cells themselves (34).
Cytogenetic karyotyping has been performed only in a limited cases and a nonrandom aberration is not identified. Two cases showed a near-tetraploid karyotype, one

with a complex chromosomal aberrations and the other one showed a 14q+ marker with unknown extramaterial on 14q32 and a dup(1)(q21q32) (30,35). Another case demonstrated complex structural and numerical aberrations, but no involvement of the 14q32 region was found by banding or fluorescence in situ hybridization (FISH) (29). Polyploidy was identified by FISH in other three cases, where additional signals for chromosomes 7, 12, and the gonosomes were observed (31). Finally, a case of THRBCL with Nijmegen breakage syndrome was found to have sporadic structural aberrations in chromosomes 7 and 14 (36).

TABLE 6.33.2

Salient Features for Laboratory Diagnosis of THRBCL

1. Flow cytometry may not demonstrate a monoclonal B-cell population.
2. Tumor cells should be positive for CD45, CD20, CD79a, BCL-6, PAX5/BSAP, BOB1, OCT2 and negative for CD15 and PU.1. CD30 is usually negative, but can be weakly positive.
3. J-chain and surface immunoglobulin are usually positive but are not important for the diagnosis.
4. The reactions to CD10, BCL-2, and EMA are variable.
5. If EBV is demonstrated by EBER or LMP1, the case should be classified as EBV-positive diffuse large B-cell lymphoma.
6. The background cells should be positive for CD3 and CD68, but negative for CD57 and CD20.
7. A follicular dendritic cell meshwork is not demonstrated by CD21.
8. Immunoglobulin heavy-chain gene rearrangement should be monoclonal.
9. BCL-2 rearrangement is seldom demonstrated.
10. BCL-1, BCL-6, and MYC gene rearrangement are negative.

The salient features for laboratory diagnosis of THRBCL are summarized in Table 6.33.2.

Clinical Manifestations

THRBCL is a clinically aggressive disease and is important to distinguish it from similar diseases, such as NLPHL and cHL, which have an indolent clinical course (12,18,37). There is a male predominance in the patient population with a median age in the fourth decade. At the time of diagnosis, most patients are in the advanced Ann Arbor stage, with multiple extranodal sites, particularly the spleen, liver, and bone marrow. Most patients have B-symptoms (fever, weight loss, and night sweats) and an international prognostic index >2 . In spite of the differences in morphology and immunophenotype, THRBCL is similar to classical diffuse large B-cell lymphoma in terms of therapeutic response and clinical outcome (37). With the CHOP-like therapy, the 3-year and 5-year overall survival rates are estimated at 50% to 64%, and 45% to 58%, respectively (12).

The current case showed a characteristic morphology of T-cell/histiocyte-rich background and scattered large tumor cells with a typical immunophenotype of THRBCL. The clinical features did not include hepatosplenomegaly, and bone marrow biopsy was not involved by lymphoma in the initial workup. However, he gradually became refractory to chemotherapy and died 15 months later due to opportunistic infection.

REFERENCES

1. Jaffe ES, Longo DL, Cossman J, et al. Diffuse B-cell lymphomas with T-cell predominance in patients with follicular lymphoma or "pseudo T-cell lymphoma". *Lab Invest*. 1984;50:27–28A.
2. Ramsay AD, Smith WJ, Isaacson PG. T-cell rich B-cell lymphoma. *Am J Surg Pathol*. 1988;12:433–443.
3. Winberg CD, Sheibani K, Burke JS, et al. T-cell rich lymphoproliferative disorders. Morphologic and immunologic differential diagnoses. *Cancer*. 1988;62:1539–1555.
4. Ng CS, Chan JKC, Hui PK, et al. Large B-cell lymphoma with a high content of reactive T-cells. *Hum Pathol*. 1989;20:1145–1154.
5. Osborne BM, Butler JJ, Pugh WC. The value of immunophenotyping on paraffin sections in the identification of T-cell rich B-cell lymphomas: lineage confirmation by JH rearrangement. *Am J Surg Pathol*. 1990;14:933–938.
6. Chital SM, Brousset P, Voigt JJ, et al. Large B-cell lymphoma rich in T-cells and simulating Hodgkin disease. *Histopathology*. 1991;19:211–220.
7. Delabie J, Vandenberghe E, Kennes C, et al. Histiocyte-rich B-cell lymphoma: a distinct clinicopathologic entity possibly related to lymphocyte predominant Hodgkin's disease, paragranuloma subtype. *Am J Surg Pathol*. 1992;16:37–48.
8. De Wolf-Peeters C, Pittaluga S. T-cell rich B-cell lymphoma: a morphological variant of a variety of non-Hodgkin's lymphomas or a clinicopathological entity? *Histopathology*. 1995;26:383–385.
9. Sun T, Susin M, Tomao F, et al. Histiocyte-rich B-cell lymphoma. *Hum Pathol*. 1997;28:1321–1324.
10. Gatter KC, Warnke RA. Diffuse large B-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:171–174.
11. De Wolf-Peeters C, Delabie J, Campo E, et al. T cell/histiocyte-rich large B-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:238–239.
12. Abramson JS. T-cell/histiocyte-rich B-cell lymphoma: biology, diagnosis, and management. *Oncologist*. 2006;11:384–392.
13. Boudova L, Torlakovic E, Delabie J, et al. Nodular lymphocyte-predominant Hodgkin lymphoma with nodules resembling T-cell/histiocyte-rich B-cell lymphoma: differential diagnosis between nodular lymphocyte-predominant Hodgkin lymphoma and T-cell/histiocyte-rich B-cell lymphoma. *Blood*. 2003;102:3753–3758.
14. Lim MS, Beaty M, Sorbara L, et al. T-cell/histiocyte-rich large B-cell lymphoma: a heterogeneous entity with derivation from germinal center B cells. *Am J Surg Pathol*. 2002;26:1458–1466.
15. Achten R, Verhoel G, Vanuytsel L, et al. Histiocyte-rich, T-cell-rich lymphoma: a distinct diffuse large B-cell lymphoma subtype showing characteristic morphologic and immunophenotypic features. *Histopathology*. 2002;40:31–45.
16. Achten R, Verhoef G, Vanuytsel L, et al. T-cell/histiocyte-rich large B-cell lymphoma: a distinct clinicopathologic entity. *J Clin Oncol*. 2002;20:1269–1277.
17. Fraga M, Garcia-Rivero A, Sanchez-Verde L, et al. T-cell/histiocyte-rich large B-cell lymphoma is a disseminated aggressive neoplasm: differential diagnosis from Hodgkin's lymphoma. *Histopathology*. 2002;41:216–229.

18. El Weshi A, Akhtar S, Mourad WA, et al. T-cell/histiocyte-rich B-cell lymphoma: clinical presentation, management and prognostic factors: report on 61 patients and review of literature. *Leuk Lymphoma*. 2007;48:1764–1773.
19. Dogan A, Burke JS, Goteri G, et al. Micronodular T-cell/histiocyte-rich large B-cell lymphoma of the spleen: histology, immunophenotype, and differential diagnosis. *Am J Surg Pathol*. 2003;27:903–911.
20. Wang SA, Olson N, Zukerberg L, et al. Splenic marginal zone lymphoma with micronodular T-cell rich B-cell lymphoma. *Am J Surg Pathol*. 2006;30:128–132.
21. Kan E, Levy I, Benharroch D. Splenic micronodular T-cell/histiocyte-rich large B-cell lymphoma. *Ann Diagn Pathol*. 2008;12:290–292.
22. Kan E, Levy I, Benharroch D. Splenic micronodular T-cell/histiocyte-rich large B-cell lymphoma: effect of prior corticosteroid therapy. *Virchow Arch*. 2009;455:337–341.
23. Rüdiger T, Gascoyne RD, Jaffe ES, et al. Workshop on the relationship between nodular lymphocyte predominant Hodgkin's lymphoma and T cell/histiocyte-rich B-cell lymphoma. *Ann Oncol*. 2002;13(suppl 1):44–51.
24. Miettinen M, Pranssila KO, Saxen E. Hodgkin's disease, lymphocytic predominance nodular. Increased risk for subsequent non-Hodgkin's lymphomas. *Cancer*. 1983;51:2293–2300.
25. Zhao FX. Nodular lymphocyte-predominant Hodgkin lymphoma or T-cell/histiocyte rich large B-cell lymphoma: the problem in "grey zone" lymphoma. *Int J Clin Exp Pathol*. 2008;1:300–305.
26. Huang JZ, Weisenburger DD, Vose JM, et al. Diffuse large B-cell lymphoma arising in nodular lymphocyte predominant Hodgkin lymphoma: a report of 21 cases from the Nebraska Lymphoma Study Group. *Leuk Lymphoma*. 2004;45:1551–1557.
27. Wang J, Sun NCJ, Chen YY, et al. T-cell/histiocyte-rich large B-cell lymphoma displays a heterogeneity similar to diffuse large B-cell lymphoma: a clinicopathologic, immunohistochemical, and molecular study of 30 cases. *Appl Immunohistochem Mol Morphol* 2005;13:109–115.
28. Chang CC, Bunyi-Teopengco E, Eshoa C, et al. CD5+ T-cell/histiocyte-rich large B-cell lymphoma. *Mod Pathol*. 2002;15:1051–1057.
29. Ozgonenel B, Savasan S, Rabah R, et al. Pediatric EBV-positive T-cell/histiocyte-rich large B-cell lymphoma with clonal cells in the bone marrow without overt involvement. *Leuk Lymphoma*. 2005;46:465–469.
30. De Leval L, Harris NL, Lampertz S, et al. T-cell/histiocyte-rich large B-cell lymphoma associated with a near-tetraploid karyotype and complex genetic abnormalities. *APMIS* 2006;114:474–478.
31. Brauner A, Kuppers R, Specker T, et al. Molecular analysis of single B cells from T-cell-rich B-cell lymphoma shows the derivation of the tumor cells from mutating germinal center B cells and exemplifies means by which immunoglobulin genes are modified in germinal center B cells. *Blood*. 1999;93:2679–2687.
32. Shimodaira S, Hidaka E, Katsuyama T. Clonal identity of nodular lymphocyte-predominant Hodgkin's disease and T-cell-rich B-cell lymphoma. *N Engl J Med*. 2000;343:1124–1125.
33. Franke S, Wlodarska I, Maes B, et al. Comparative genomic hybridization pattern distinguishes T-cell/histiocyte-rich B-cell lymphoma from nodular lymphocyte predominance Hodgkin's lymphoma. *Am J Pathol*. 2002;161:1851–1867.
34. Monti S, Savage KJ, Hutok JL, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. *Blood*. 2005;105:1851–1861.
35. La Starza R, Aventin A, Falzetti D, et al. 14q+ chromosome marker in a T-cell-rich B-cell lymphoma. *J Pathol*. 1996;178:227–231.
36. Paulli M, Viglio A, Boveri E, et al. Nijmegen breakage syndrome-associated T-cell-rich B-cell lymphoma: case report. *Pediatr Dev Pathol*. 2003;3:264–270.
37. Bouabdallah R, Mounier N, Guettier C, et al. T-cell/histiocyte-rich large B-cell lymphomas and classical diffuse large B-cell lymphomas have similar outcome after chemotherapy: a matched-control analysis. *J Clin Oncol*. 2003;21:1271–1277.

CASE 34

Primary Mediastinal (Thymic) Large B-Cell Lymphoma

CASE HISTORY

A 16-year-old girl was admitted to the hospital because of low-grade fever, respiratory distress, and orthopnea for several days. After admission, a chest roentgenogram showed a large mediastinal mass accompanied by a left pleural effusion. Echocardiogram revealed significant compromise of venous return and decrease in myocardial contractility. These findings were consistent with the superior vena cava syndrome. An abdominal sonogram and computed tomography demonstrated multiple intrarenal lesions. The bone marrow and the central nervous system were, however, not involved. A biopsy of the mediastinal

mass was taken, and a diagnosis of lymphoma was established.

She received several courses of chemotherapy and radiation therapy. After each course, the mediastinal mass shrank for a short period of time but recurred repeatedly. The patient finally underwent high-dose chemotherapy and autologous bone marrow transplant. After such treatment, chest roentgenogram and computed tomography showed no mediastinal abnormality, and an abdominal sonogram demonstrated disappearance of the kidney lesions. One year after bone marrow transplantation, the patient showed no evidence of tumor recurrence.

FLOW CYTOMETRY FINDINGS

Lymph node biopsy: Large cell gate: B-cell markers: IgG 1%, IgA 0%, IgM 2%, κ 1%, λ 1%, CD19 98%, CD20 99%, HLA-DR 100%. T-cell markers: CD3 3%, CD5 4%, CD7 2%. Monocyte markers: CD11c 13%, CD14 9% (Fig. 6.34.1 not the same case).

Small cell gate: B-cell markers: IgG 0%, IgA 0%, IgM 7%, κ 5%, λ 3%, CD19 23%, CD20 25%, HLA-DR 77%. T-cell markers: CD3 88%, CD5 92%, CD7 84%.

IMMUNOFLUORESCENT STAIN

Terminal deoxynucleotidyl transferase (TdT) was negative.

DISCUSSION

Primary mediastinal (thymic) large B-cell lymphoma (PMLBCL) was first described by Lichtenstein et al. (1) in 1980. It is the most frequently found non-Hodgkin lymphoma in the mediastinum, mainly in the anterior mediastinum. The tumor cells are derived from the medullary B cells in the thymus. Recent evidence suggests that PMLBCL is closely related to the nodular sclerosis subtype of classical Hodgkin lymphoma (cHL) because of the similarity in clinical, morphologic, immunophenotypic, and molecular

genetic aspects between these two tumors. In fact, there are cases of mediastinal tumors that are intermediate between these two neoplasms morphologically and immunologically and are, therefore, designated mediastinal gray zone lymphoma (2,3). PMLBCL is included in the Revised European-American Lymphoma classification as a separate entity under B-cell neoplasm (4). In the 2001 World Health Organization (WHO) classification, this tumor is classified as a subtype of diffuse large B-cell lymphoma (DLBCL) (5). DLBCL is composed of a heterogeneous group of B-cell lymphomas with large tumor cells, but PMLBCL is a distinct clinicopathological entity clearly distinguished from other large B-cell lymphomas in light of modern technologies (6). In the 2008 WHO classification, PMLBCL remains as a subgroup of DLBCL (7), but the mediastinal gray zone lymphoma is classified under the title of B-cell lymphoma, unclassifiable with features intermediate between DLBCL and cHL (8).

Morphology

The tumor cells of PMLBCL may show great variation both in size and in shape among different patients or within the same patient (5). However, a typical case usually shows large tumor cells with clear cytoplasm and extensive fibrosis that compartmentalizes the tumor cells (Figs. 6.34.2 and 6.34.3) (9). Therefore, in the early literature, this tumor was described as sclerosing, large clear

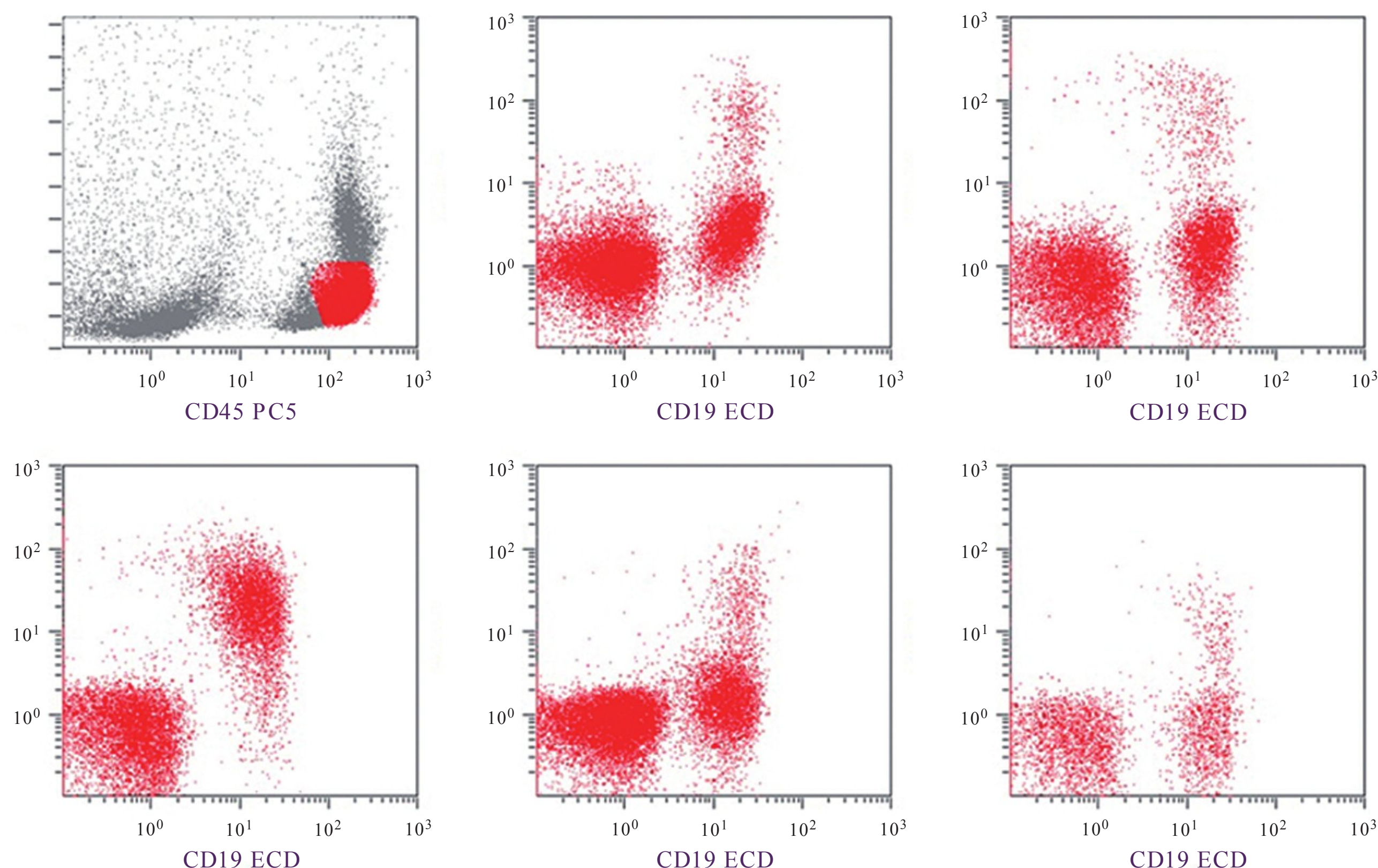


FIGURE 6.34.1 Typical flow cytometric immunophenotype shows positive reactions to CD19 and CD23, but negative reactions to κ , λ , CD21, and CD10. SS, side scatter; PC5, phycoerythrin–cyanin 5; FITC, fluorescein isothiocyanate; ECD, phycoerythrin-Texas Red; PE, phycoerythrin.

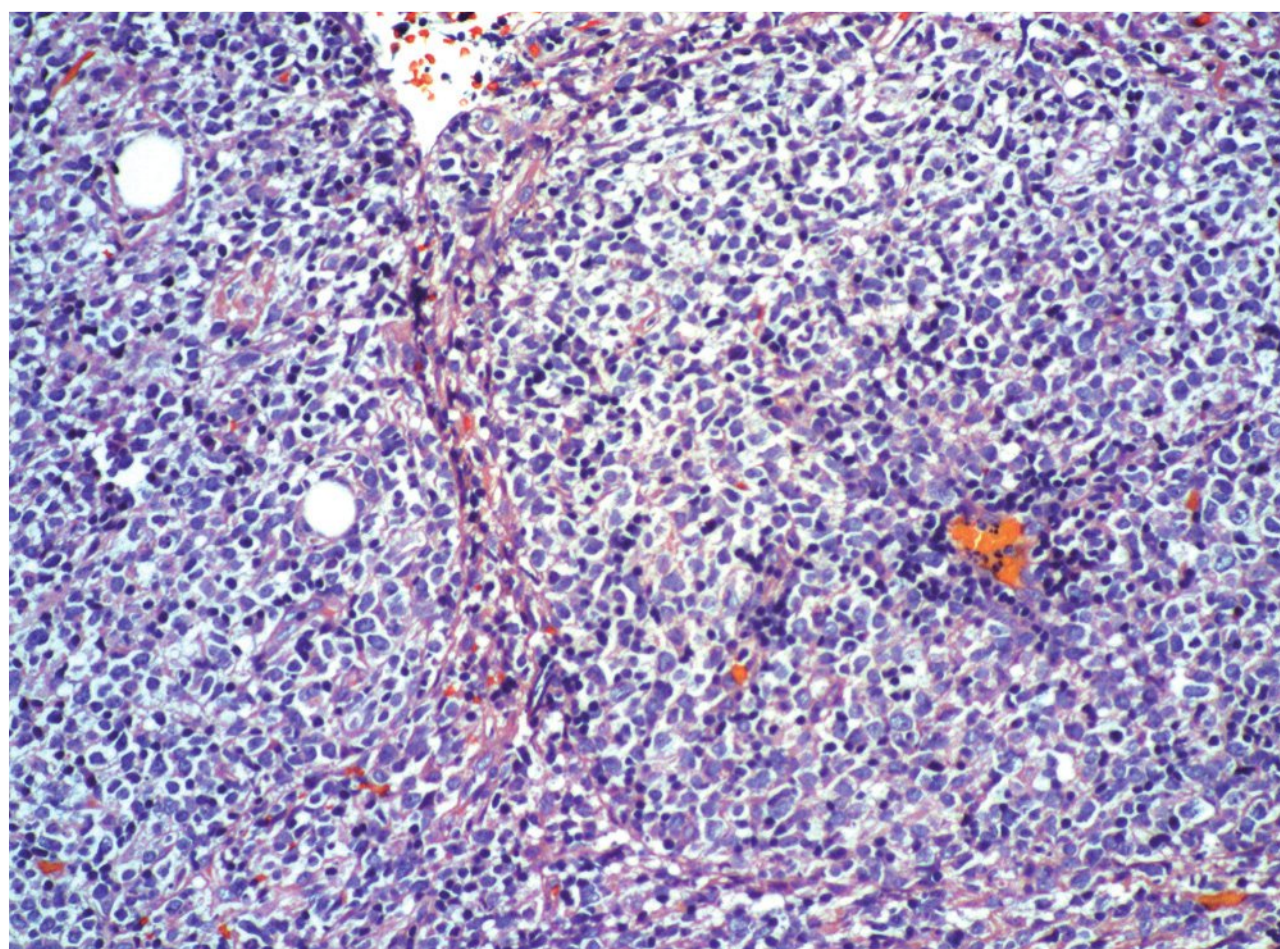


FIGURE 6.34.2 Mediastinal biopsy reveals diffuse, large clear cell infiltration separated by fibrous bands (compartmentalization). Hematoxylin and eosin, 20× magnification.

cell lymphoma. However, some reported cases showed medium-sized tumor cells without clear cytoplasm and/or no sclerosis (10,11). The tumor cells can also be cleaved, noncleaved, centroblastic, or immunoblastic. As mentioned before, some tumors may be difficult to distinguish from Hodgkin lymphoma in morphology (e.g., the presence of Reed-Sternberg-like cells), and others may show an immunophenotype mimicking Hodgkin lymphoma (CD30+, CD15+) (2,3). These variations are probably due to the fact that some tumors in the mediastinum may be secondary/metastatic and that mediastinal B-cell lymphomas may be derived from different tissues, namely, the thymus and the lymph node (12). Evidence supporting the thymic origin of this tumor is based on the fact that epithelium-lined cysts, expanded thymic lobules, Hassall corpuscles (Fig. 6.34.4), or isolated epithelial cells are found in some cases (9). With the help of the cytokeratin stain, thymic remnants

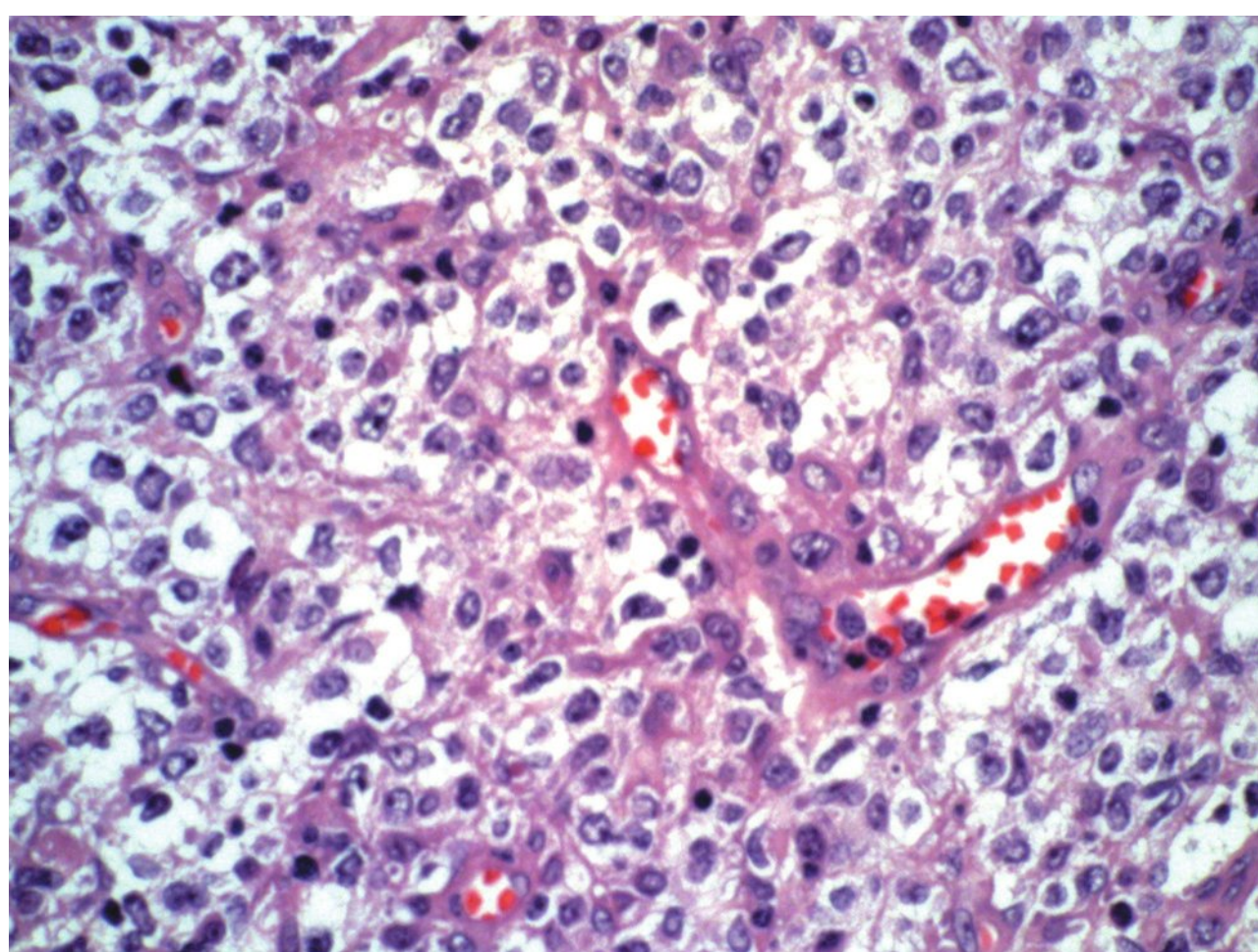


FIGURE 6.34.3 Mediastinal biopsy shows large clear cells arranged in a lobular pattern, mimicking seminoma. Hematoxylin and eosin, 40× magnification.

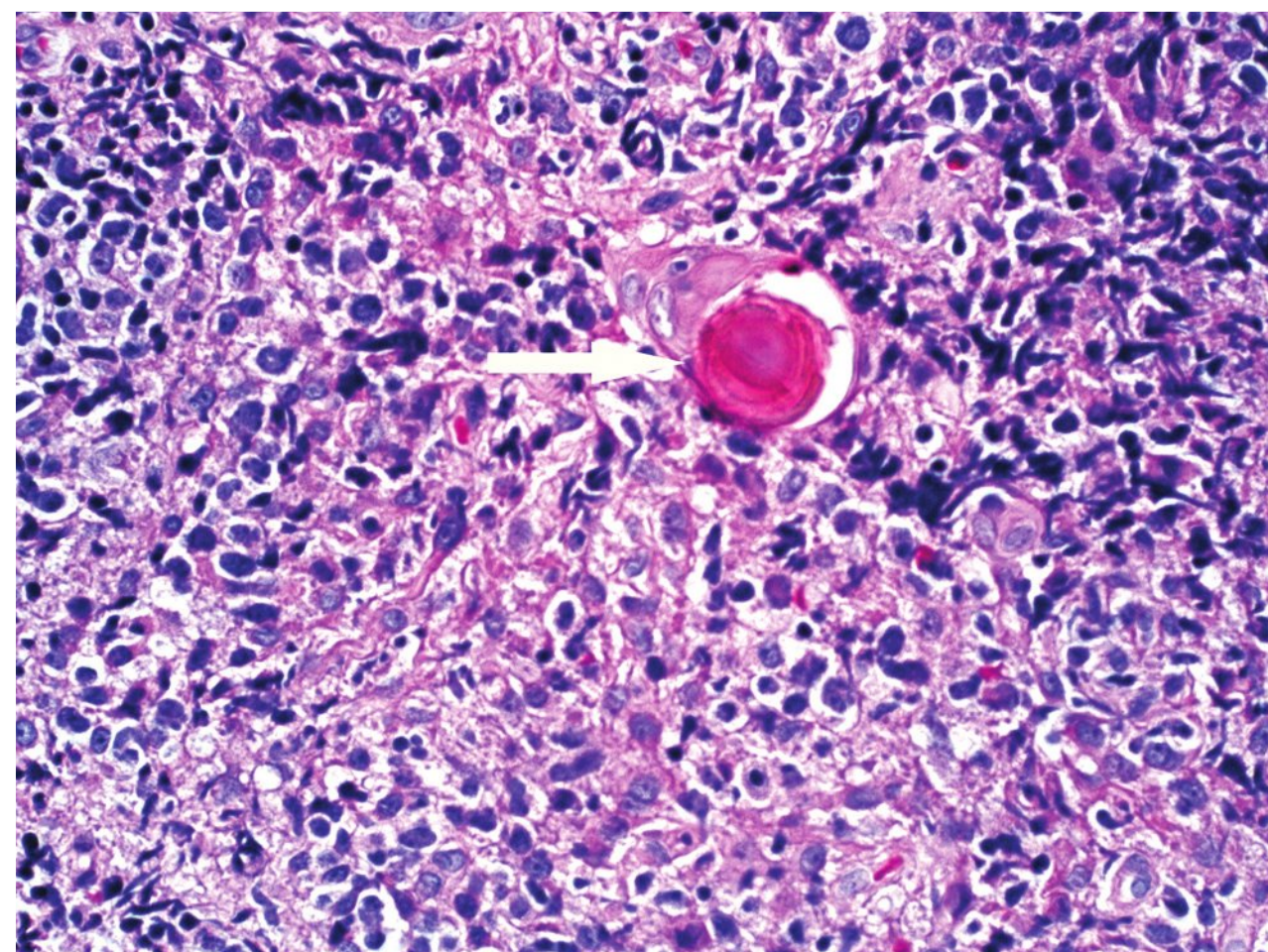


FIGURE 6.34.4 Mediastinal biopsy shows a Hassall corpuscle among the tumor cells indicating its derivative from the thymic medulla. Hematoxylin and eosin, 40× magnification.

or fibrous tissue reminiscent of thymus is detected in one third to one half of specimens. The immunophenotype of this tumor is also similar to that of the medullary B cells in the thymus, which will be discussed later.

The heterogeneity in morphology is also because the diagnostic criteria used in various series are different. Therefore, the Nebraska Lymphoma Study Group defined a “primary” MLBCL as “a mediastinal mass of at least 5 cm in maximum dimensions, with no extramediastinal mass larger than that in the mediastinum” (10). With this definition, most secondary tumors spread from other locations can be excluded. However, there are also cases of composite lymphomas with feature of both PMLBCL and cHL, and mediastinal sequential lymphomas, in which PMLBCL with relapses as cHL or vice versa (2). These cases cannot be explained by metastatic tumors. The diagnostic morphologic features of MLBCL are summarized in Table 6.34.1.

Immunophenotype

The most important markers for the immunophenotype are the B-cell markers. CD20 and CD19 are consistently positive either by immunohistochemistry (Fig. 6.34.5) or by flow cytometric analysis. Among other B-cell markers, CD22 and CD23 are positive in the vast majority of PMLBCL, but CD10 and CD21 (C3d receptor) are negative. CD23 is expressed in a subpopulation of large, dendritic cells (asteroid cells) among thymic B cells (13), and the absence of CD21 is also characteristic for the medullary thymic B cells (14–17). The hallmark of the immunophenotype in PMLBCL is the absence of surface Igs, which is also seen in medullary thymic B cells (14–17).

Surface Ig on human B cells is noncovalently associated with a disulfide-linked heterodimer (designated CD79), which is composed of mb-1 (CD79a) and B29 (CD79b). Because the expression of surface Ig requires these polypeptide chains, the surface Ig/CD79 complex is also called the B-cell antigen receptor (BCR) complex. One of the characteristic features in PMLBCL is the discordant expression of Ig and its associated molecule mb-1/CD79a. In other words, CD79a is expressed despite the absence of surface Igs (14).

TABLE 6.34.1

Characteristic Morphologic Features of Mediastinal Large B-Cell Lymphoma

Histologic pattern	Diffuse large cell infiltration encircled by fibrous bands
Cytology	Large cell with clear cytoplasm
Specific features	Bulky mediastinal tumor with the above histologic and cytologic features

In the same study, this immunophenotype (Ig[−]/CD79a⁺) was found in only five cases of follicular lymphoma among 110 cases of various types of lymphoma studied. Another discordant phenomenon is the expression of three transcription factors, OCT2, BOB.1, and PU.1, which are required for germinal center formation and Ig production (2).

Other B-cell-related antigens, including bcl-2, bcl-6, HLA-DR, B-cell specific activator protein PAX5/BSAP, and MUM1/IRF4, have also been reported positive in PMLBCL cases (6,18). The plasma cell-associated antigen CD38 is negative in this tumor (6). There is also a defect in the expression of HLA class I and II molecules (12,15). The recent finding of expression of three gene products, MAL, REL, and FIGI, as well as the tumor necrosis factor receptor-associated factor (TRAF-1), is considered relatively specific to PMLBCL (19–22). In addition, two other markers, p63 (TP73L) and growth factor receptor-bound protein 2 (Grb2), are found in PMLBCL with high frequencies but are usually negative for cHL (23,24). On the other hand, the expression of cyclin E is highly predictive for cHL and consistently negative for PMLBCL (25).

Among mediastinal tumors, the differential diagnoses include lymphoblastic lymphoma, Hodgkin lymphoma, thymoma, and seminoma. Lymphoblastic lymphoma is

usually of T-cell phenotype, but PMLBCL is positive for B-cell markers and consistently negative for T-cell markers (CD3, CD5, CD43, CD45RO) (9,26). In addition, CD10, the common acute lymphocytic leukemia (ALL) antigen that is positive in some lymphoblastic lymphomas is negative in PMLBCL. The absence of TdT in the current case also contradicts the diagnosis of lymphoblastic lymphoma. However, TdT has not been included in the study of PMLBCL in the literature.

Similar to PMLBCL, cHL also shows no surface immunoglobulin, and 80% PMLBCL cases express CD30 with weak and heterogeneous pattern (2,3,15,19,27). These findings may cause some confusion in the differential diagnosis between these two tumors. However, CD45 is positive and CD15 is generally negative in PMLBCL, whereas the Reed–Sternberg cells usually show the opposite reactions. In addition, B-cell transcription factors, MAL, REL, FIGI, TRAF-1, p63, and Grb2, are negative in cHL. Nevertheless, molecular genetic evidence shows that MLBCL and Hodgkin lymphoma may be transformable to each other, and transitional cases (mediastinal gray zone lymphoma) have been reported (2,3). As mentioned before, these cases are now categorized as B-cell lymphoma, unclassifiable, with features between DLBCL and cHL (8). The immunophenotypic differences between PMLBCL, cHL, and intermediate PMLBCL/cHL are listed in Table 6.34.2.

Thymoma usually expresses the lymphoblastic lymphoma phenotype, but its reaction to cytokeratin is most helpful in excluding PMLBCL. The lobular pattern and large clear cells in seminoma may sometimes be mistaken as PMLBCL, and human chorionic gonadotropin b has been detected in a case of PMLBCL (28); therefore, seminoma should always be included in the differential diagnosis. However, PMLBCL shows extensive B-cell staining, whereas seminoma is positive for placental alkaline phosphatase (Fig. 6.34.6).

The current case showed typical demographic findings and characteristic histology of PMLBCL. Immunophenotyping revealed the presence of B-cell antigens and absence of surface Ig, T-cell antigens, and TdT. The renal lesion might represent metastatic tumor, and kidney is a common site for PMLBCL involvement. Therefore, it is a typical case of PMLBCL. The diagnostic features for PMLBCL are summarized in Table 6.34.3.

Comparison of Flow Cytometry and Immunohistochemistry

The basic immunophenotype of MLBCL is that of a B-cell antigen-positive and surface Ig-negative lymphoma. This immunophenotype can be demonstrated by both flow cytometry and immunohistochemistry. However, immunohistochemistry can correlate various markers—B-cell, surface Ig, T-cell, CD30, CD15, and placental alkaline phosphatase—with the tumor cells, so this technique is more helpful in establishing a definitive diagnosis. There are also many new markers (MAL, FIGI, REL, TRAF-1, p63, Grb2, and cyclin E) that can only be demonstrated by immunohistochemistry.

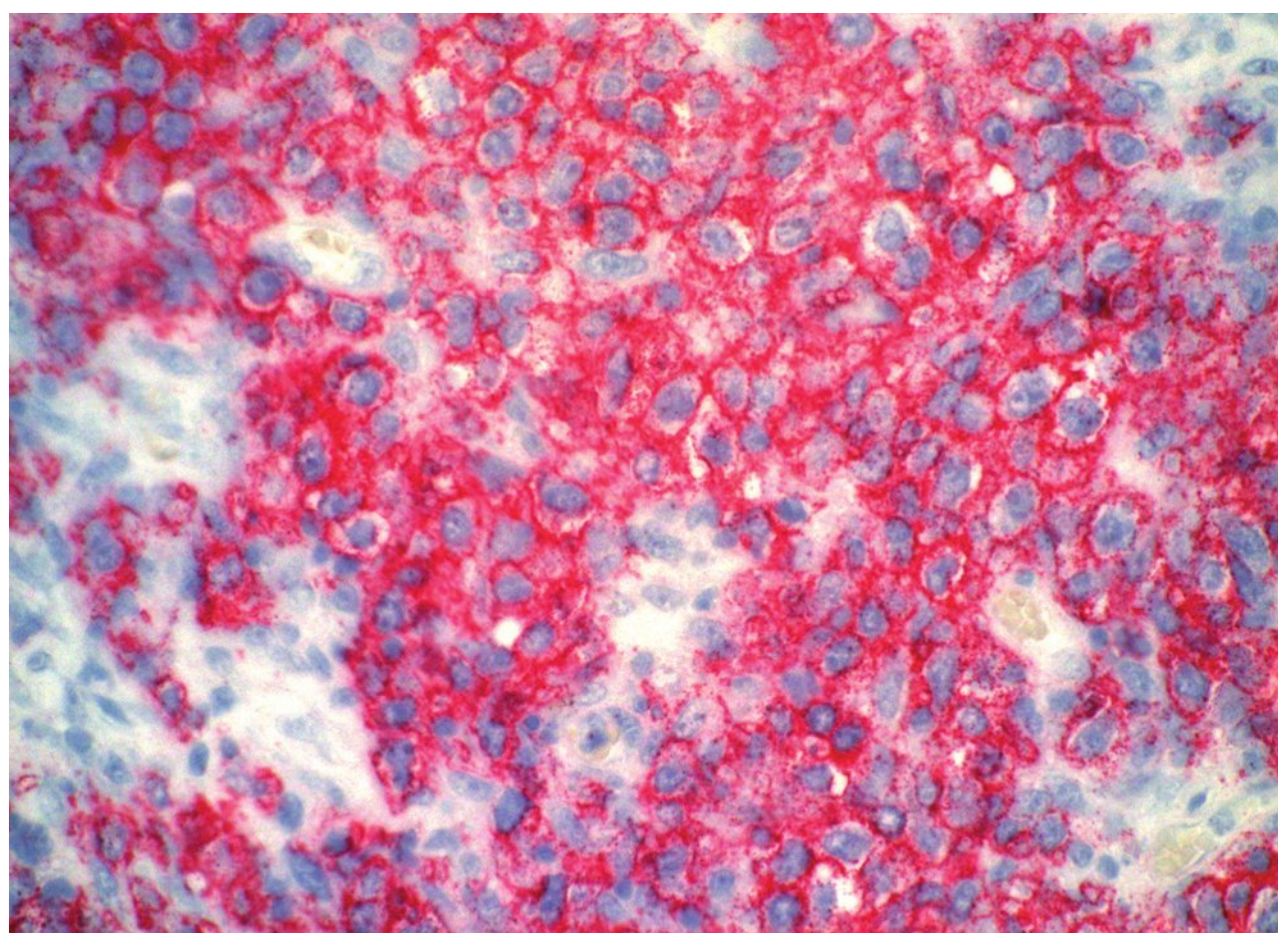


FIGURE 6.34.5 Mediastinal biopsy reveals positive CD20 staining of the tumor cells. Immunoalkaline phosphatase, 40× magnification.

TABLE 6.34.2			
Immunophenotypic Differences between PMLBCL, cHL, and Intermediate PMLBCL/cHL			
	PMLBCL	cHL	PMLBCL/ cHL
B-cell antigens	Positive	Negative	Positive
PAX5	Positive	Positive	Positive
B-cell transcription factors	Positive	Negative	Positive
Surface immunoglobulin	Negative	Negative	Positive
MUM1/IRF4	Positive	Positive	Positive
bcl-6	Positive	Negative	Variable
bcl-2	Positive	Negative	N/A
CD30	Weakly positive	Positive	Positive
CD15	Occasionally positive	Positive	Positive
CD45	Positive	Negative	Positive
MAL	Positive	Negative	Positive
FIGI	Positive	Negative	Negative
REL	Positive	Negative	Positive
TRAF-1	Positive	Negative	Negative
p63	Positive	Negative	Positive
Cyclin E	Negative	Positive	Negative
Grb2	Positive	Negative	Positive
EBV	Negative	Positive	Negative

B-cell antigens, CD19, CD20, CD22, CD79a; B-cell transcription factors, BOB1, OCT2, PU.1; cHL, classical Hodgkin lymphoma; EBV, Epstein–Barr virus; Grb2, growth factor receptor-bound protein 2; N/A, data not available; PMLBCL, primary mediastinal large B-cell lymphoma; PMLBCL/cHL, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and cHL; TRAF-1, tumor necrosis factor receptor-associated factor 1.

Molecular Genetics

Although PMLBCL shows no surface Ig, rearrangement of heavy-chain and light-chain genes has been regularly detected. This discrepancy reflects nonproductive Ig gene rearrangement (14). However, unlike cHL, PMLBCL does not have deleterious mutation of IgH gene; therefore, other molecular mechanisms must be involved. The possible mechanisms include down-regulation of Ig internal enhancer activity (29) and post-transcriptional blockage (30).

Multiple molecular genetic abnormalities, such as point mutation of the MYC oncogene and p53 suppressor gene, amplification of the REL protooncogene, and gains of 9p, 12q, and Xq, have been detected (16,18,31). The more specific findings are the gain of chromosome 2p15, which is associated with amplification of the transcription factor c-REL and genome amplification of 9q24, which involves the JAK2 gene (20).

The molecular characteristics of PMLBCL have been gradually established by current studies with gene expression profiling. The two major findings are (i) that MLBCL is distinctly different from other DLBCLs and (ii) that it closely resembles Hodgkin lymphomas.

The most specific molecular marker for PMLBCL is the expression of the MAL gene, and its messenger RNA can be identified by Northern blot and reverse transcription-polymerase chain reaction (32,33). The MAL protein can also be detected in PMLBCL cells but not in other DLBCLs. As MAL expression is restricted to a minor subpopulation of thymic medullary B cells, these findings provide further support for the thymic B-cell origin of PMLBCL (33).

In the earlier literature, the absence of BCL-6 rearrangement/mutation in PMLBCL was considered a major distinction between PMLBCL and other DLBCLs (6,17,34,35). However, a recent study claimed that 54% of MLBCL cases showed hypermutation of BCL-6, but the hypermutation sites and mutational spectrum of BCL-6 in PMLBCL differed from those found in DLBCLs and follicular lymphoma (36). Another recent study also found that more than half of PMLBCL cases displayed BCL-6 gene mutation, which usually occurred along with variable region of Ig heavy-chain (IgV_H) gene mutations or MUM1/IRF4 expression (37). Akasaka et al. (38) reported that BCL-6 translocation involving the IgV_H gene was associated with a favorable prognosis, whereas those involving a non-Ig partner were not.

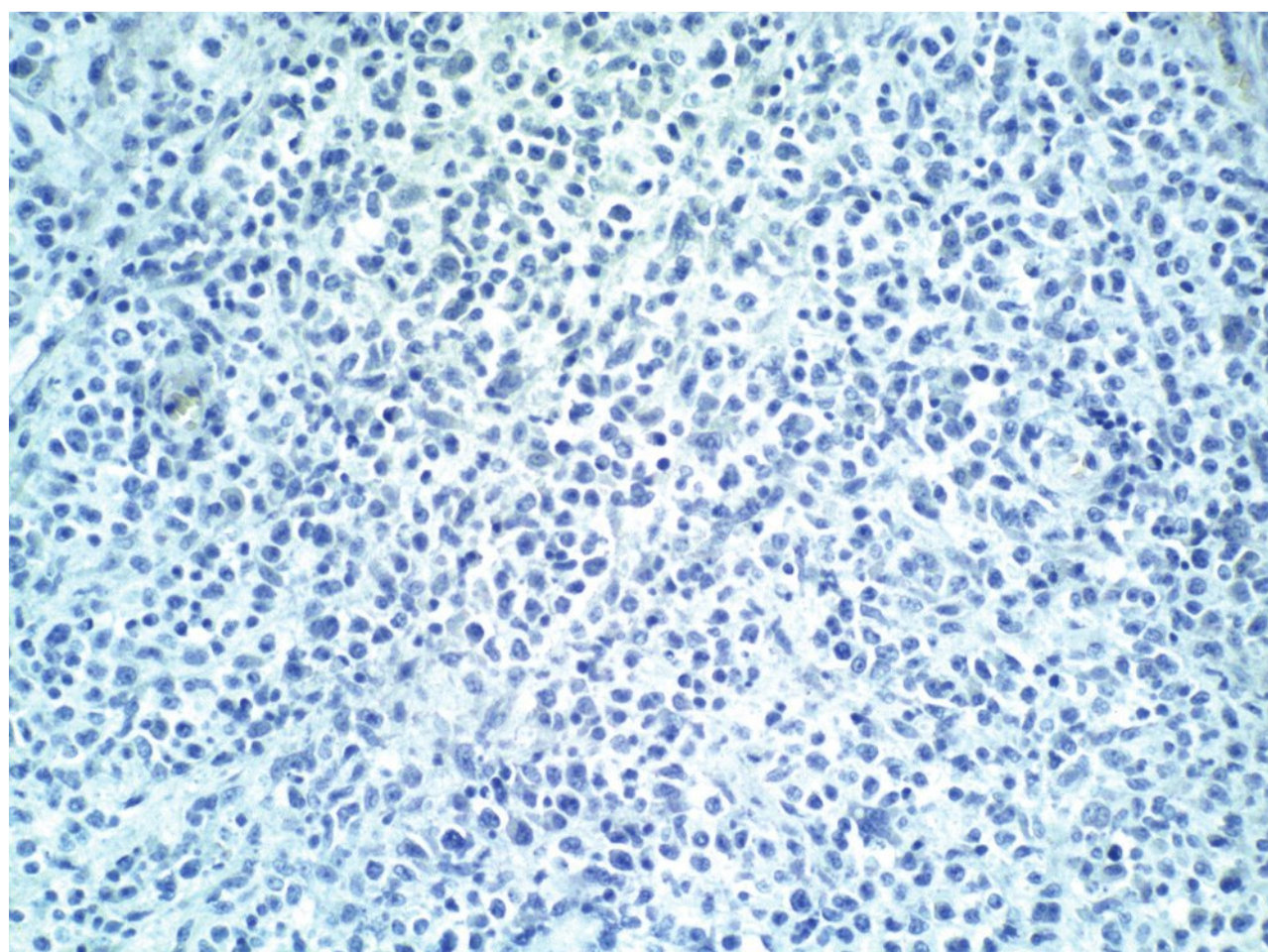


FIGURE 6.34.6 Mediastinal biopsy reveals negative reaction of the tumor cells to placental alkaline phosphatase. Immunoperoxidase, 10× magnification.

The absence of BCL-2 rearrangement/mutation in PMLBCL is still considered an important characteristic distinguishing PMLBCL from other DLBCLs (6,17,35). Paradoxically, bcl-2 protein overexpression has been demonstrated in PMLBCL cases in the same frequency as in other DLBCLs (34,35). Other negative molecular findings in PMLBCL include the absence of BCL-1 rearrangements, the ras oncogene, and the Epstein–Barr virus (EBV) genome (17).

Gene expression profiling studies have found that the signature gene expression profile of PMLBCL is more closely related to classic Hodgkin lymphoma than to other DLBCLs (39). In one such study, more than one third of the genes that were more highly expressed in PMLBCL cases than in other DLBCL cases were expressed in cases of Hodgkin lymphoma (40). Other molecular features sharing between PMLBCL and cHL include decrease of BCR

pathway signaling, constitutive NFκB activation, activation of the cytokine-JAK-STAT pathway, and aberrant activation of P13K/AKT pathway (19).

Clinical Manifestations

The clinical features of PMLBCL are so characteristic that they usually help the pathologists arrive at an accurate diagnosis. In a study conducted by the Non-Hodgkin's Lymphoma Classification Project, 85% of the expert pathologists made the correct diagnosis when clinical history was available, whereas only 58% of them arrived at the same diagnosis without the history (41).

The characteristic clinical history is that of a young woman (age: 15 to 45 years) showing a bulky mediastinal mass (>10 cm in diameter in three quarters of patients) and pulmonary symptoms, such as cough, chest pain, and dyspnea (9,42). The tumor usually spreads locally, invading lungs, superior vena cava, pleura, pericardium, and chest wall. About one half of the patients manifest superior vena cava syndrome, and one third have pleural and pericardial effusions (26,43,44). Distant spread seldom occurs. When present, the tumor usually involves internal organs rather than lymph nodes. The most commonly involved organ is the kidney, but invasion of the liver, adrenal glands, pancreas, ovaries, and brain also has been reported (26,45,46).

Nonspecific laboratory findings include moderate-to-marked increase in the level of serum lactate dehydrogenase and a normal or only slightly elevated serum level of b₂-microglobulin (47). As the tumor is usually bulky, the latter finding is unusual. A possible explanation is that b₂-microglobulin is the light-chain component of HLA-I, which is deficient in PMLBCL (9).

Therapeutic failure was frequently encountered in the early reported cases of PMLBCL. However, since the current application of aggressive chemotherapy, radiation therapy, and/or bone marrow transplant, the prognosis of PMLBCL has improved (9,43,44). According to the Non-Hodgkin's Lymphoma Classification Project, the 5-year overall survival is 50% and the 5-year failure-free survival is 48% (41). A recent study of 141 consecutive patients with a median follow-up of 10.9 years in the Memorial Sloan-Kettering Cancer Center showed an event-free survival and overall survival of 50% and 66%, respectively (48). The European study groups showed 5-year survival rates of 39% to 96% (25). It is paradoxical that PMLBCL is cytogenetically closer to cHL than DLBCL and yet the effective therapeutic regimen for PMLBCL is that of DLBCL and not cHL (25,35).

TABLE 6.34.3

Salient Features for Laboratory Diagnosis of PMLBCL

1. Absence of surface immunoglobulin
2. B-cell antigen phenotype: CD10–, CD19+, CD20+, CD21–, CD22+, CD23+, CD79a+, bcl-2+, bcl-6+, HLA-DR+, PAX5+, BOB1+, OCT2+, and PU.1+
3. New markers: MAL, REL, FIGI, TRAF-1, p63, and Grb2
4. Large cell size as determined by forward light scatter
5. T-cell markers are negative.
6. Immunoglobulin gene rearrangement is present.
7. Expression of MAL gene

Grb2, growth factor receptor-bound protein 2; TRAF-1, tumor necrosis factor receptor-associated factor.

REFERENCES

1. Lichtenstein AK, Levine A, Taylor OR, et al. Primary mediastinal lymphoma in adults. *Am J Med.* 1980;68:506–514.
2. Traverse-Glehen A, Pittaluga S, Gaulard P, et al. Mediastinal gray zone lymphoma: the missing link between classic Hodgkin lymphoma and mediastinal B-cell lymphoma. *Am J Surg Pathol.* 2005;29:1411–1421.
3. Poppema S, Kluiver JL, Atayaar C, et al. Report: workshop on mediastinal grey zone lymphoma. *Eur J Haematol Suppl.* 2005;66:45–52.

4. Harris NL, Jaffe ES, Stein H, et al. A revised European-American Classification of Lymphoid Neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
5. Banks PM, Warnke RA. Mediastinal (thymic) large B-cell lymphoma. In: Jaffe E, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:175–176.
6. Pileri SA, Dirnhofer S, Went PH, et al. Diffuse large B-cell lymphoma: one or more entities? Present controversies and possible tools for its subclassification. *Histopathology*. 2002;41:482–509.
7. Gaulard P, Harris NL, Pileri SA, et al. Primary mediastinal (thymic) large B-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:250–251.
8. Jaffe ES, Stein H, Swerdlow SH, et al. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:267–268.
9. Aisenberg AC. Primary large cell lymphoma of the mediastinum. *Semin Oncol*. 1999;26:251–258.
10. Abou-Elella AA, Weisenburger DD, Vose JM, et al. Primary mediastinal large B-cell lymphoma: a clinicopathologic study of 43 patients from the Nebraska Lymphoma Study Group. *J Clin Oncol*. 1999;17:784–790.
11. Paulli M, Strater J, Gianelli U, et al. Mediastinal B-cell lymphoma. A study of its histomorphologic spectrum based on 109 cases. *Hum Pathol*. 1999;30:178–187.
12. Chadburn A, Frizzera G. Mediastinal large B-cell lymphoma vs classic Hodgkin's lymphoma. *Am J Clin Pathol*. 1999;112:155–158.
13. Calaminici M, Piper K, Lee AM, et al. CD23 expression in mediastinal large B-cell lymphomas. *Histopathology*. 2004;45:619–624.
14. Kanavaros P, Gaulard P, Charlotte F, et al. Discordant expression of immunoglobulin and its associated molecule mb-1/CD79a is frequently found in mediastinal large B cell lymphomas. *Am J Pathol*. 1995;146:735–741.
15. Falini B, Venturi S, Martelli M, et al. Mediastinal large B-cell lymphoma. Clinical and immunohistological findings in 18 patients treated with different third-generation regimens. *Br J Haematol*. 1995;89:780–789.
16. Hofmann WJ, Momburg F, Moller P. Thymic medullary cells expressing B-lymphocyte antigens. *Hum Pathol*. 1988;19:1280–1287.
17. Tsang P, Cesarman E, Chadburn A, et al. Molecular characterization of primary mediastinal B-cell lymphoma. *Am J Pathol*. 1996;148:2017–2025.
18. Pileri SA, Zinzani PL, Gaidano G, et al. Pathobiology of primary mediastinal B-cell lymphoma. *Leuk Lymphoma*. 2003;44(suppl 3):S21–S26.
19. Carbone A, Gloghini A, Aiello A, et al. B-cell lymphomas with features intermediate between distinct pathologic entities. From pathogenesis to pathology. *Hum Pathol*. 2010;41:621–631.
20. Boleti E, Johnson PWM. Primary mediastinal B-cell lymphoma. *Hematol Oncol*. 2007;25:157–163.
21. Martelli M, Ferreri AJM, Johnson P. Primary mediastinal large B-cell lymphoma. *Crit Rev Oncol Hematol*. 2008;68:256–263.
22. Rodig SJ, Savage KJ, Harris NL, et al. Expression of TRAF1 and nuclear C-Rel distinguishes primary mediastinal large B-cell lymphoma from nodal diffuse large B-cell lymphoma. *Mod Pathol*. 2006;19:244a.
23. Zamò A, Malpeli G, Scarpa A, et al. Expression of TP73L is a helpful diagnostic marker of primary mediastinal large B-cell lymphomas. *Mod Pathol*. 2005;18:1448–1453.
24. Miles RR, Mankey CC, Seiler CE III, et al. Expression of Grb2 distinguishes classical Hodgkin lymphomas from primary mediastinal B-cell lymphomas and other diffuse large B-cell lymphomas. *Hum Pathol*. 2009;40:1731–1737.
25. Hoeller S, Zihler D, Zlobec I, et al. BOB.1, CD79a and cyclin E are the most appropriate markers to discriminate classical Hodgkin's lymphoma from primary mediastinal large B-cell lymphoma. *Histopathology*. 2010;56:217–228.
26. Lazzarino M, Orlandi E, Paulli M, et al. Primary mediastinal B-cell lymphoma with sclerosis. An aggressive tumor with distinctive clinical and pathologic features. *J Clin Oncol*. 1993;11:2306–2313.
27. Higgins JP, Warnke RA. CD30 expression is common in mediastinal large B-cell lymphoma. *Am J Clin Pathol*. 1999;112:241–247.
28. Fraternali-Orcinoni G, Falini B, Quaini F, et al. b-HCG aberrant expression in primary mediastinal large B-cell lymphoma. *Am J Surg Pathol*. 1999;23:717–721.
29. Ritz O, Leithauser F, Hasel C, et al. Downregulation of internal enhancer activity contributes to abnormally low immunoglobulin expression in the MedB-1 mediastinal B-cell lymphoma cell line. *J Pathol*. 2005;205:336–348.
30. Loddenkemper C, Anagnostopoulos I, Hummel M, et al. Differential Emu enhancer activity and expression of BOB.1/OBF.1, Oct2, PU.1, and immunoglobulin in reactive B-cell populations, B-cell non-Hodgkin lymphomas, and Hodgkin lymphomas. *J Pathol*. 2004;202:60–69.
31. Joos S, Otano-Joos MI, Ziegler S, et al. Primary mediastinal (thymic) B-cell lymphoma is characterized by gains of chromosomal material including 9p and amplification of the REL gene. *Blood*. 1996;87:1571–1578.
32. Anagnostopoulos I, Dallenbach F, Stein H. Diffuse large cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:855–913.
33. Copie-Bergman C, Plonquet A, Alonso MA, et al. MAL expression in lymphoid cells: further evidence for MAL as a distinct molecular marker of primary mediastinal large B-cell lymphomas. *Mod Pathol*. 2002;15:1172–1180.
34. Cazals-Hatem D, Lepage E, Brice P, et al. Primary mediastinal large B-cell lymphoma: a clinicopathologic study of 141 cases compared with 916 nonmediastinal large B-cell lymphomas, a GELA ("Groupe d'Etude des Lymphomes de l'Adulte") study. *Am J Surg Pathol*. 1996;20:877–888.
35. von Besien K, Kelta M, Bahaguna P. Primary mediastinal B-cell lymphoma: a review of pathology and management. *J Clin Oncol*. 2001;19:1855–1864.
36. Malpeli G, Barbi S, Moore PS, et al. Primary mediastinal B-cell lymphoma: hypermutation of the BCL6 gene targets motifs different from those in diffuse large B-cell and follicular lymphomas. *Hematologica*. 2004;89:1091–1099.
37. Pileri SA, Gaidano G, Zinzani PL, et al. Primary mediastinal B-cell lymphoma (PMBL): high frequency of BCL-6 mutations and consistent expression of the transcription factors Oct-2 and BOB.1 in the absence of immunoglobulin expression. *Am J Pathol*. 2003;162:243–253.
38. Akasaka T, Ueda C, Kurata M, et al. Nonimmunoglobulin (non-Ig)/BCL-6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. *Blood*. 2000;96:2907–2909.

39. Calvo KR, Traverse-Glehen A, Pittaluga S, et al. Molecular profiling provides evidence of primary mediastinal large B-cell lymphoma as a distinct entity related to classic Hodgkin lymphoma: implications for mediastinal gray zone lymphomas as an intermediate form of B-cell lymphoma. *Adv Anat Pathol*. 2004;11:227–238.
40. Rosenwald A, Wright G, Leroy K, et al. Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. *J Exp Med*. 2003;198:851–862.
41. The Non-Hodgkin's Lymphoma Classification Project. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. *Blood*. 1997;89:3909–3918.
42. Andreopoulou H, Pectasides D, Dimopoulos MA, et al. Primary mediastinal large B-cell lymphoma: clinical study of a distinct clinical entity and treatment outcome in 20 patients: review of the literature. *Am J Clin Oncol*. 2004;27:312–316.
43. Lazzarino M, Orlandi E, Paulli M, et al. Treatment outcome and prognostic factors for primary mediastinal (thymic) B-cell lymphoma. A multicenter study of 106 patients. *J Clin Oncol*. 1997;15:1646–1653.
44. Zinzani PL, Bendandi M, Frezza G, et al. Primary mediastinal B-cell lymphoma with sclerosis. Clinical and therapeutic evaluation of 22 patients. *Leuk Lymphoma*. 1996;21:311–316.
45. Kirn D, Mauch P, Shaffer K, et al. Large-cell and immunoblastic lymphoma of the mediastinum. Prognosis and pathologic features in 57 patients. *J Clin Oncol*. 1993;11:1336–1343.
46. Todeschini G, Ambrosetti A, Meneghini V, et al. Mediastinal large B-cell lymphoma with sclerosis. A Clinical study of 21 patients. *J Clin Oncol*. 1990;8:804–808.
47. Rodriguez J, Pugh WC, Romaguera JE, et al. Primary mediastinal large cell lymphoma is characterized by an inverted pattern of large tumoral mass and low beta-2 microglobulin levels in serum and frequently elevated levels of serum lactate dehydrogenase. *Ann Oncol*. 1994;5:847–849.
48. Hamlin PA, Portlock CS, Straus DJ, et al. Primary mediastinal large B-cell lymphoma: optimal therapy and prognostic factor analysis in 141 consecutive patients treated at Memorial Sloan Kettering from 1980–1999. *Br J Haematol*. 2005;130:691–699.

CASE 35

Intravascular Large B-Cell Lymphoma

CASE HISTORY

A 70-year-old man presented with dementia for several months. The patient had no history of exposure to radiation or toxic chemicals. He also had no history of malignancy. Physical examination showed a disoriented old man with no focal neurological deficit. He had no enlarged lymph node and no hepatosplenomegaly. There was no skin rash present. Peripheral blood examination revealed mild anemia, but the total leukocyte count and platelet count were within normal ranges. No atypical lymphoid cells were detected in the blood. The blood chemistry panel was unremarkable except for elevation of lactate dehydrogenase (LDH). Cerebral magnetic resonance imaging showed patchy white matter inclusions. A brain biopsy was taken (Figs. 6.35.1 and 6.35.2).

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Immunohistochemical stains showed that the tumor cells were positive for CD45 and CD20 (Fig. 6.35.3), but negative for CD3 (Fig. 6.35.4) and pancytokeratin. Epstein—Barr virus encoded RNA (EBER) hybridization was also negative.

DISCUSSION

Intravascular large B-cell lymphoma (IVLBCL) was first described by Pflieger and Tappeiner in Germany in 1959

as angioendotheliomatosis proliferans systemica, because they believed that the tumor cells were derived from the endothelium (1). It was not until the mid 1980s that the tumor was finally identified as lymphoma by immunophenotyping (2). Therefore, many terms have been used in the interim, including neoplastic angioendotheliosis, malignant angioendotheliomatosis, intravascular lymphomatosis, angioendotheliotropic (intravascular) lymphoma, and

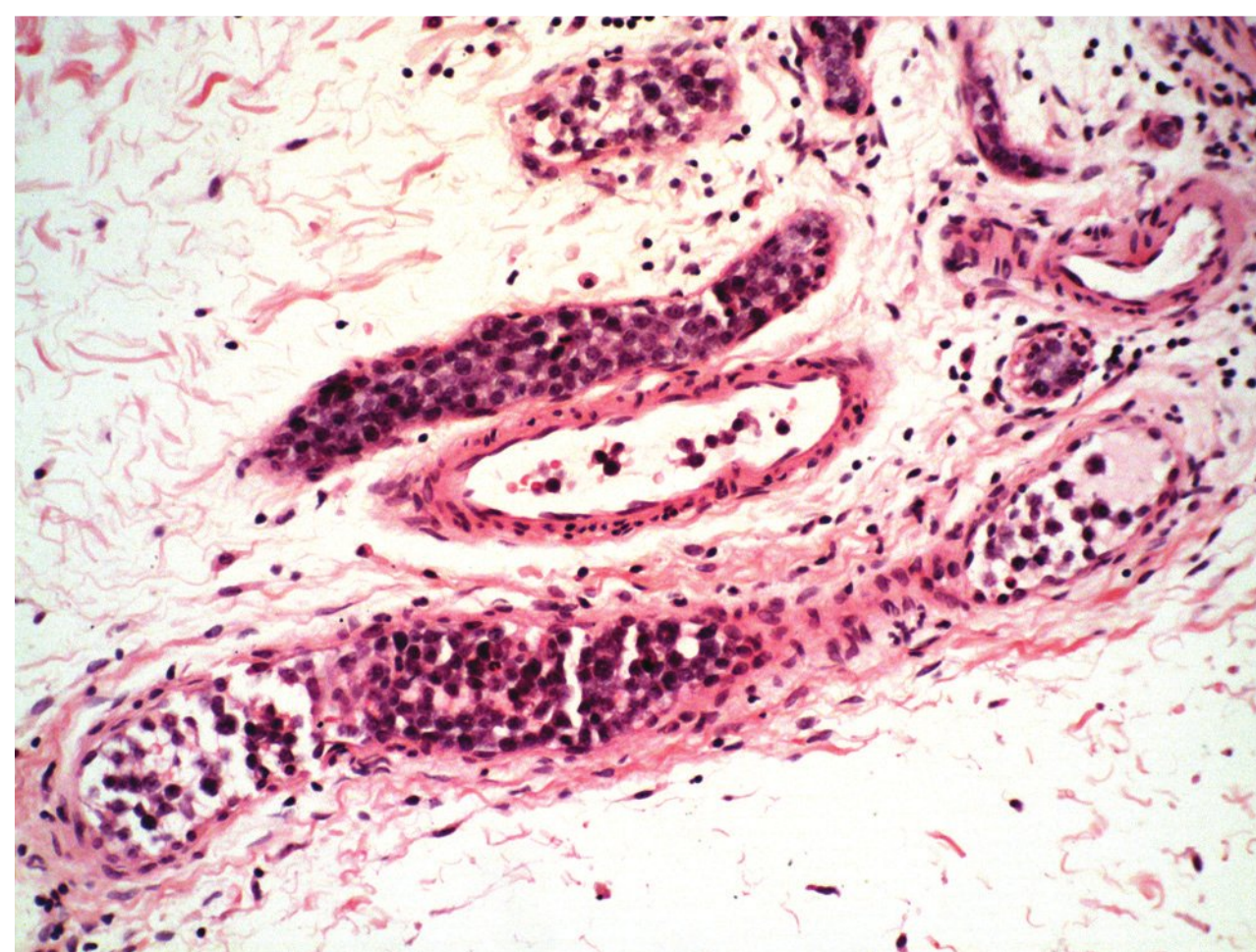


FIGURE 6.35.1 Brain biopsy shows intravascular lymphoma cell infiltration in several small blood vessels and capillaries. H&E, × 20.

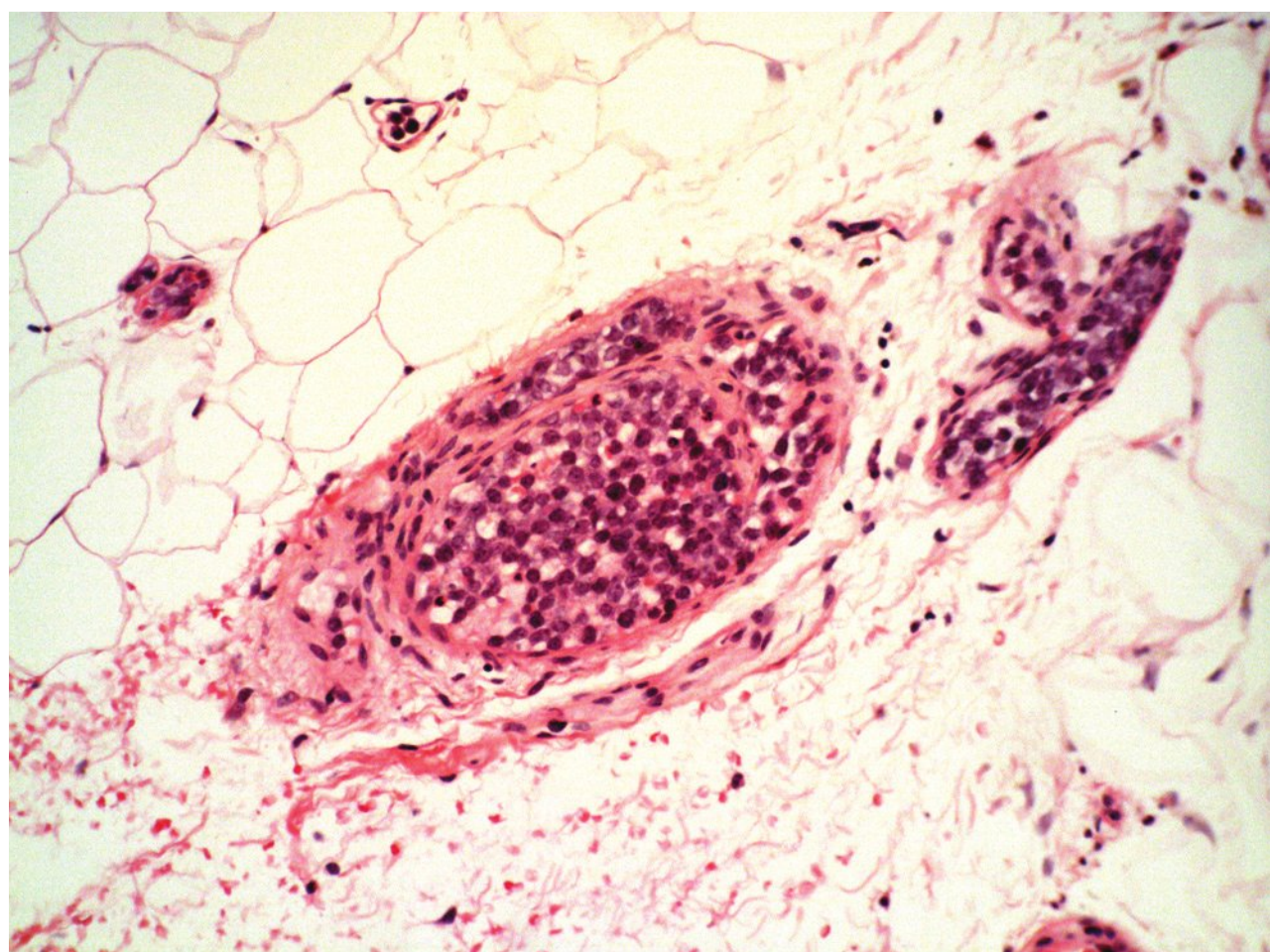


FIGURE 6.35.2 Another view of the brain biopsy shows similar features. H&E, $\times 20$.

angiotropic large-cell lymphoma (1,3). The World Health Organization (WHO) designates it as IVLBCL and classifies it as a subtype of diffuse large B-cell lymphoma (4). However, some authors still prefer the name intravascular lymphoma to encompass the rare subtypes with T cell or natural killer (NK) cell origin (1).

MORPHOLOGY

IVLBCL is defined as a subtype of diffuse large B-cell lymphoma confined exclusively to the lumina of small blood vessels, mainly capillaries, without the involvement of the surrounding parenchymal tissues (1,4,5). Although IVLBCL has been detected in practically all internal organs in autopsy cases, lymph nodes are characteristically spared in the majority of cases.

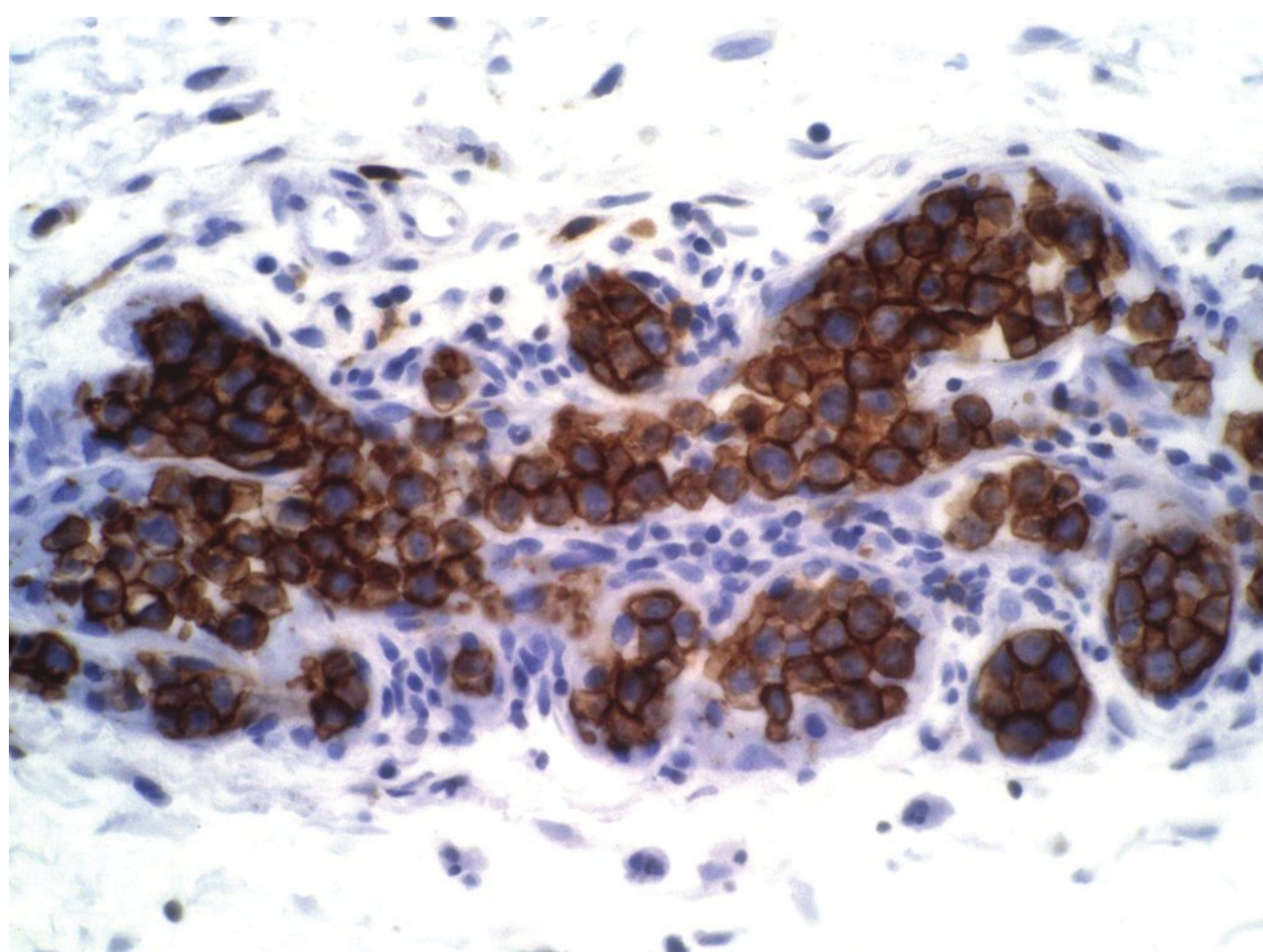


FIGURE 6.35.3 The intravascular lymphoma cells stain positive for CD20. Immunoperoxidase, $\times 40$.

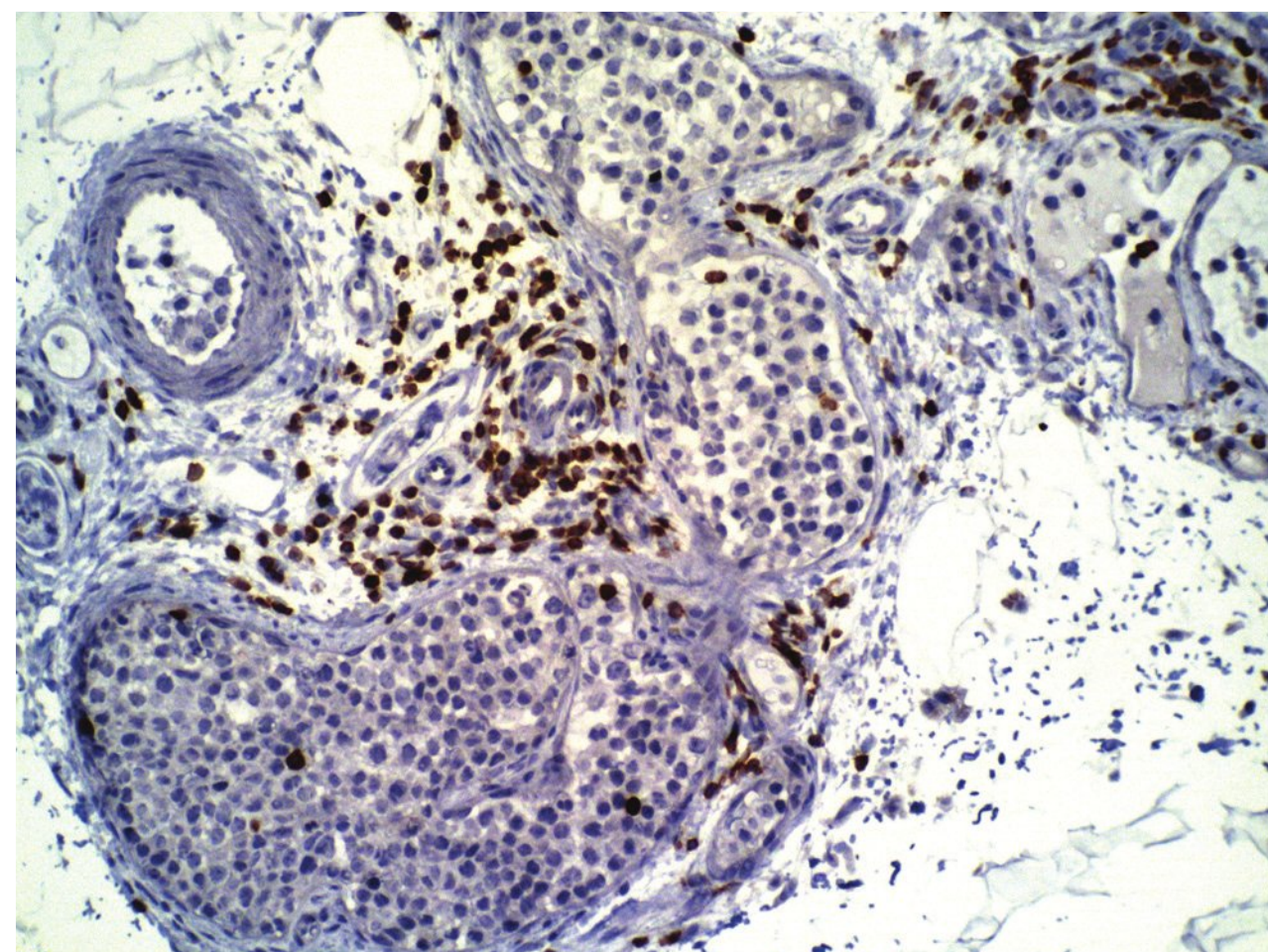


FIGURE 6.35.4 The perivascular lymphocytes are positive for CD3. Immunoperoxidase, $\times 20$.

As implied by its designation, the tumor cells are usually large with prominent nucleoli. High mitotic rate may be demonstrated in some cases (4). However, small cells, cleaved cells, and clumpy chromatin pattern also have been reported (5). As a result of tumor cell obstruction, fibrin thrombi, hemorrhages, and necrosis of focal tissues may occur. Although intravascular tumor cell infiltration is the hallmark of this disease, extravascular involvement has been reported in a few cases, and extravascular infiltration may develop during the course of the disease (1,6). Furthermore, IVLBCL may be associated with nodal-based lymphoma, some of which may show diffuse large B-cell morphology, or de novo large B-cell lymphoma in the brain with IVLBCL in other organs (5,6). In those cases, it is hard to determine if IVLBCL represents a distinct clinical entity or dissemination of other lymphomas.

Patients from Western countries mainly show involvement of the central nervous system and the skin. In the brain, IVLBCL may involve the meninges, subarachnoid space, cortex, deep gray matter structures, and white matter (7). Examination of the cerebrospinal fluid may demonstrate elevation of protein with occasional detection of tumor cells (1,8). The frequency of skin lesions varies from 14% to 60% in different reported (1). However, the actual frequency can be much higher, because IVLBC can be identified at random biopsy of apparently normal skin tissue in some studies (9). In about one quarter of cases, cutaneous lesion is the only clinical presentation and in this cutaneous variant, the prognosis is significantly better than the systemic IVLBCL (1,8). The skin lesions tend to favor the proximal extremities, lower abdomen, and submammary areas (10). Histologically, the tumor cells are seen in the lumina of small blood vessels in the dermis and/or subcutaneous tissue. Vascular occlusion by fibrin thrombi and perivascular plasma cell infiltration may be present.

The Asian variant differs from the classical IVLBCL in that pathology is mainly seen in the liver, spleen, and the bone marrow (11–14). In the liver, the tumor cells usually show intrasinusoidal distribution. However, focal,

extravascular intraparenchymal spread has also been noted in some cases (5). In the spleen, red pulp involvement is the common pattern, which may also be seen in other types of lymphoma and leukemia. Therefore, an extrasplenic presence of intravascular lymphoma cells is required for the diagnosis of splenic involvement by IVLBCL (5). In the bone marrow, intrasinusoidal involvement of lymphoma cells with frequent hemophagocytosis is the most characteristic feature in the Asian variant (5,12,13).

In the kidneys, lymphoma cells reveal a peculiar infiltration pattern in the glomerular capillaries in addition to the classical intravascular proliferation in interstitial vessels (5,15). In the lungs, the tumor cells not only present in capillaries, but also in small veins and arterioles. Frequently, the tumor cells are enmeshed in platelet and fibrin meshwork, causing intra-alveolar hemorrhage, fibrinous exudates, and collections of alveolar macrophages (3,16). Some cases may also have diffuse thickening of the alveolar septa.

Immunophenotype

The tumor cells express all common B-cell markers, including CD19, CD20, CD22, and CD79a (1). On the basis of the expression of CD10, BCL-6, and MUM1/IRF4, the tumor cells are further classified into the germinal center B-cell-like (GCB) and the non-GCB subtypes (17). GCB is CD10+, BCL-6+/-, MUM1/IRF4-, while non-GCB is CD10-, BCL-6+/-, MUM1/IRF4+ (6,13). Most cases of IVLBCL are of the non-GCB or non-GCB subtype. BCL-2 expression in the non-GCB subtype confers a poor prognosis (18). CD5 marker in de novo diffuse large B-cell lymphoma usually predicts an aggressive clinical course, but CD5 positivity does not influence the prognosis of IVLBCL (12,13). Ki-67 may be positive in a high percentage (70% to 80%) of tumor cells (8). Even in cases where high mitotic rates are not present, Ki-67 is useful to confirm the high proliferative activity of IVLBCL (5). EBER is usually negative in IVLBCL (12).

Up to 2008, <20 cases of intravascular T-cell lymphoma had been reported (19). In the 12 cases summarized by Cerroni, 11 cases are positive for CD3, 8 cases are double negative for both CD4 and CD8, 7 cases are positive for CD56, 11 cases are positive for cytotoxic proteins, and 8 cases are positive for Epstein—Barr virus by EBER1 (19). Until 2008, <10 cases of intravascular NK cell lymphoma had been reported (20,21). In the six cases summarized by Nakamichi, all cases are positive for CD3, CD56, and TIA. All but one case show presence of EBV and all five cases with CD4/CD8 studies show double negative results for these markers (20).

The predilection of the lymphoma cells for capillary endothelium is likely related to the homing receptors expressed on the surface of the tumor cells. Aberrant expression of CD11a and CD49d (VLA-4) on IVLBCL cell is the likely mechanism. It is because these adhesion molecules would enable tumor cells to home to CD54 (CD11a ligand) and CD106 (CD49d ligand), which are expressed on endothelial cell surfaces (22). Another possible mechanism is the lack of CD29 (b₁ integrin) and CD54 (ICAM-1) on the tumor cells, because these adhesion molecules facilitate transvascular migration (23).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is seldom used because of the rarity of tumor cells and because most specimens are fixed before the tumor cells are discovered. Immunohistochemistry provides direct morphologic and immunophenotypic correlation in the small number of intravascular tumor cells and helps to establish the diagnosis. Immunohistochemical staining with CD10, BCL-2, BCL-6, and MUM1/IRF4 may further subclassify the tumor for prognostic prediction.

Molecular Genetics

Cytogenetic studies have been performed on <20 cases. Most cases are of the Asian variant, but there are no marked differences between the Asian and classical cases in terms of karyotyping. In summarizing all reported cases in the literature, Khoury et al. (24) found that most cases showed a complex karyotype, which frequently includes abnormal chromosomes 1, 3, 6, 11, 14, and 18. This group also identified cryptic translocations der(10)t(10;22), der(17)t(17;22), and balanced t(11;14) in two cases by multicolor karyotyping. Shimazaki et al. (25) reported that 8p21 and 19q13 were characteristic chromosomal abnormality in their seven cases. Most reports, however, considered aberrations in chromosomes 1, 6, and 18 most important (12,24,26).

Tsukadaira et al. suggested that the frequent involvement of 1p aberration may be associated with the apoptotic regulatory gene BCL-10 (26). However, the BCL-10 gene demonstrated in the extranodal B-cell lymphoma usually involves 1p22, which has not been demonstrated in the IVLBCL cases that showed 1p abnormalities. In the literature, 10/17 cases (59%) demonstrated complete or partial deletions of chromosome 6, with 6q21-23 being the most commonly deleted region (24). Deletion of 6q21, 6q23, 6q25-27, 6q15, and 6q11-13 is also commonly seen in other diffuse large B-cell lymphomas (27). These sites are considered to be the location of the tumor suppressor genes, and their deletion could be the mechanism of tumorigenesis (27). Trisomy 18 or duplication of the long arm of chromosome 18 has been found in 7/17 (41%) of reported IVLBCL cases, but not as a primary alteration (24). This region is known to contain the BCL-2 oncogene at 18q21.3, as demonstrated in follicular lymphoma or other diffuse large B-cell lymphomas. However, this association has not yet been established in IVLBCL cases (24). In conclusion, the karyotypic abnormalities demonstrated in IVLBCL cases are similar to other lymphomas, particularly other diffuse large B-cell lymphomas. However, the association with oncogenes that are seen commonly in B-cell lymphomas, such as BCL-1, BCL-2, BCL-6, and MYC, has not yet been established.

The current case involved only the brain with the demonstration of neoplastic B-cells in the cerebral blood vessels and is thus a case of classical IVLBCL. Skin and bone marrow involvement were not demonstrated. The salient features for laboratory diagnosis of IVLBCL are listed in Table 6.35.1.

TABLE 6.35.1

Salient Features for Laboratory Diagnosis of IVLBCL

1. Intravascular proliferation and/or sinusoidal involvement of lymphoma cells.
2. Bone marrow may show hemophagocytosis.
3. Immunophenotype: Monoclonal B-cell population with positive B-cell markers (CD19, CD20, CD22, CD79a) and immunoglobulin light-chain restriction demonstrated by immunohistochemistry or flow cytometry. CD5 is positive in some cases.
4. Immunologic subtypes:
 - Non-GCB: CD10[−], BCL-6[±], MUM1/IRF4⁺
 - GCB: CD10⁺, BCL-6[±], MUM1/IRF4[−]
5. Cytogenetics: Karyotypic aberrations are mostly seen in chromosomes 1, 6, and 18.

Clinical Manifestations

IVLBCL is an aggressive tumor with rapid dissemination and affects elderly patients (1,4,10). Therefore, the prognosis is poor and most patients die in months after the diagnosis. Clinically, many patients have fever, weight loss, and night sweats. Anemia and thrombocytopenia are also common findings when bone marrow is involved. Laboratory findings include elevation of LDH, b2 microglobulin, and erythrocyte sedimentation rate. Serum prostatic acid phosphatase (PAP) is also elevated and PAP can be detected on the tumor cells by immunohistochemistry but not other lymphomas (28). Therefore, it is proposed that serum PAP should be used for early diagnosis and for therapeutic monitoring of IVLBCL. Clinical symptoms are extremely heterogeneous, depending on the organs involved. In spite of the intravascular infiltration, leukemic presentation is rare in patients with IVLBCL (29).

In the Western countries, the central nervous system and skin are most frequently involved, which is designated the classical type of IVLBCL (10,30,31). However, in Asian countries, mainly Japan, patients often show involvement of the bone marrow, liver, and spleen that is designated Asian variant (9,11–14). Lymph node involvement is rare. One characteristic finding in these patients is hemophagocytosis demonstrated in the bone marrow; thus, this entity was also called diffuse large B-cell lymphoma associated with hemophagocytic syndrome or malignant histiocytosis-like B-cell lymphoma (11,12). Some cases may not have morphologic evidence of hemophagocytosis; in those cases, the diagnosis of hemophagocytic syndrome is based on the clinical presentation of fever, hepatosplenomegaly, and thrombocytopenia (5). The third clinical subtype of IVLBCL variant is the cutaneous variant, which is seen invariably in female patients with only skin involvement and shows consistently a better outcome than other clinical subtypes (10).

Nevertheless, recent reports indicate that bone marrow and hepatosplenic involvement is also common in patients

from the Western countries (10). On the other hand, skin involvement is also often found in Asian patients. In fact, random skin biopsy in Asian patients showed a high frequency of skin involvement (9). In a report from China, the clinical symptoms of their patients are more similar to those of the Western countries rather than those of Japan (8). Lung and kidney involvement was considered rare, but current reports found a high frequency of involvement in these organs and the authors even suggested random biopsies of lung and kidney as a tool for the diagnosis of IVLBCL (3,15,16).

REFERENCES

1. Zuckerman D, Selim R, Hochberg E. Intravascular lymphoma: the oncologist's "great imitator". *Oncologist*. 2006;11:496–502.
2. Sheibani K, Battifora H, Winberg CD, et al. Further evidence that "malignant angioendotheliomatosis" is an angiotropic large-cell lymphoma. *N Engl J Med*. 1986;314:943–948.
3. Ko YH, Han JH, Go JH, et al. Intravascular lymphomatosis: a clinicopathological study of two cases presenting as an interstitial lung disease. *Histopathology*. 1997;31:555–562.
4. Nakamura S, Ponzoni M, Campo E. Intravascular large B-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:252–253.
5. Ponzoni M, Ferreri AJM, Campo E, et al. Definition, diagnosis, and management of intravascular large B-cell lymphoma: Proposals and perspectives from an international consensus meeting. *J Clin Oncol*. 2007;25:3168–3173.
6. Imai H, Shimada K, Shimada S, et al. Comparative clinicopathological study of primary CNS diffuse large B-cell lymphoma and intravascular large B-cell lymphoma. *Pathol Int*. 2009;59:431–437.
7. Williams RL, Meltzer CC, Smirniotopoulos JG, et al. Cerebral MR imaging in intravascular lymphomatosis. *Am J Neuroradiol*. 1998;19:427–431.
8. Kong YY, Dai B, Sheng WQ, et al. Intravascular large B-cell lymphoma with cutaneous manifestations: a clinicopathologic, immunophenotypic and molecular study of three cases. *J Cutan Pathol*. 2009;36:865–870.
9. Matsue K, Asada N, Takeuchi M, et al. A clinicopathological study of 13 cases of intravascular lymphoma: experience in a single institution over a 9-yr period. *Eur J Haematol*. 2007;80:236–244.
10. Ferreri AJM, Campo E, Seymour JF, et al. Intravascular lymphoma: clinical presentation, natural history, management and prognostic factors in a series of 38 cases, with special emphasis on the 'cutaneous variant'. *Br J Haematol*. 2004;127:173–183.
11. Murase T, Nakamura S, Tashiro K, et al. Malignant histiocytosis-like B-cell lymphoma, a distinct pathologic variant of intravascular lymphomatosis: a report of five cases and review of the literature. *Br J Haematol*. 1997;99:656–664.
12. Murase T, Nakamura S, Kawauchi K, et al. An Asia variant of intravascular large B-cell lymphoma: clinical, pathological and cytogenetic approaches to diffuse large B-cell lymphoma associated with haemophagocytic syndrome. *Br J Haematol*. 2000;111:826–834.
13. Murase T, Yamaguchi M, Suzuki R, et al. Intravascular large B-cell lymphoma (IVLBCL): a clinicopathological study of 96 cases with special reference to the immunophenotypic heterogeneity of CD5. *Blood*. 2007;109:478–485.

14. Murase T, Nakamura S. An Asian variant of intravascular lymphomatosis: an updated review of malignant histiocytosis-like B-cell lymphoma. *Leuk Lymphoma*. 1999;33:459–473.
15. Niitsu N, Okamura D, Takahashi N, et al. Renal intravascular large B-cell lymphoma with early diagnosis by renal biopsy: A case report and review of the literature. *Leuk Res*. 2009;33:728–730.
16. Yousem SA, Colby TV. Intravascular lymphomatosis presenting in the lung. *Cancer*. 1990;65:349–353.
17. Hans CP, Weisenburger DD, Greiner TC, et al. Confirmation of the molecular classification of diffuse large cell lymphoma by immunohistochemistry using tissue microarray. *Blood*. 2004;103:275–282.
18. Iqbal J, Neppalli VT, Wright G, et al. BCL2 expression is a prognostic marker for the activated B-cell like type of diffuse large B-cell lymphoma. *J Clin Oncol*. 2006;24:961–968.
19. Cerroni L, Massone C, Kutzner H, et al. Intravascular large T-cell or NK-cell lymphoma: a rare variant of intravascular large cell lymphoma with frequent cytotoxic phenotype and association with Epstein-Barr virus infection. *Am J Surg Pathol*. 2008;32:891–898.
20. Nakamichi N, Fukuhara S, Aozasa K, et al. NK-cell intravascular lymphomatosis—a mini-review. *Eur J Haematol*. 2008;81:1–7.
21. Wu H, Said JW, Ames ED, et al. First reported cases of intravascular large cell lymphoma of the NK cell type. Clinical, histologic, immunophenotypic, and molecular features. *Am J Clin Pathol*. 2005;123:603–611.
22. Kanda M, Suzumiya J, Ohshima K, et al. Intravascular large cell lymphoma: clinicopathological, immuno-histochemical and molecular genetic studies. *Leuk Lymphoma*. 1999;34:569–580.
23. Ponzoni M, Arrigoni G, Gould VE, et al. Lack of CD29 (beta integrin) and CD54 (ICAM-1) adhesion molecules in intravascular lymphomatosis. *Hum Pathol*. 2000;31:220–226.
24. Khoury H, Lestou V, Gascoyne RD, et al. Multicolor karyotyping and clinicopathological analysis of three intravascular lymphoma cases. *Mod Pathol*. 2003;16:716–724.
25. Shimazaki C, Inaba T, Shimura K, et al. B-cell lymphoma associated with haemophagocytic syndrome: a clinical immunological and cytogenetic study. *Br J Haematol*. 1999;104:672–679.
26. Tsukadaira A, Okubo Y, Ogasawara H, et al. Chromosomal aberrations in intravascular lymphomatosis. *Am J Clin Oncol*. 2002;25:178–181.
27. Nanjangud V, Rao PH, Hegde A, et al. Spectral karyotyping identifies new rearrangements, translocations, and clinical associations in diffuse large B-cell lymphoma. *Blood*. 2002;99:2554–2561.
28. Seki K, Miyakoshi S, Lee GH, et al. Prostatic acid phosphatase is a possible tumor marker for intravascular large B-cell lymphoma. *Am J Surg Pathol*. 2004;28:1384–1388.
29. Xanthopoulos V, Galanopoulos AG, Patrakis G, et al. Intravascular B-cell lymphoma with leukemic presentation: case report and literature review. *Eur J Haematol*. 2007;80:177–181.
30. Baehring JM, Longtine J, Hochberg FH. A new approach to the diagnosis and treatment of intravascular lymphoma. *J Neurooncol*. 2003;61:237–248.
31. DiGiuseppe JA, Nelson WG, Seifter EH, et al. Intravascular lymphomatosis: A clinicopathologic study of 10 cases and assessment of response to chemotherapy. *J Clin Oncol*. 1994;12:2573–2579.

CASE 36

Burkitt Lymphoma/Leukemia

CASE HISTORY

A 79-year-old man presented to the emergency room with a 7-day history of increasing bowel distention and mild abdominal pain. His past medical history did not reveal relevant symptoms. Physical examination showed distended abdomen with signs of free fluid. Liver and spleen were not palpable. A computed tomography (CT) scan of the abdomen demonstrated free air with a scant amount of free fluid in the abdominal cavity. A paracentesis yielded 50 mL of bloody fluid, which grew *Clostridium difficile*. A Gastrografin enema demonstrated a cecal mass without extravasation of contrast.

The patient was diagnosed with perforated cecum and underwent an exploratory laparotomy 5 days after admission. A cecal mass was resected, and an ileostomy was performed. Biopsy of the cecal mass was diagnosed as a lymphoma. After the operation, the patient had persistent absence of bowel function, but a CT scan of the abdomen did not detect intra-abdominal abscess. However, the patient's clinical condition deteriorated, and he had

progressive worsening of oxygenation saturation, hemodynamic instability, and respiratory distress. The patient died 2 weeks after admission.

FLOW CYTOMETRY FINDINGS

B-cell markers: CD19 73%, CD19/CD5 0%, CD20 73%, CD23 9%, CD10 72%, FMC-7 36%, k 0%, l 74%, CD19/k 4%, CD19/k 54%. T-cell markers: CD3 28%, CD5 25%, CD7 25%. Activation antigen: CD25 58% (Fig. 6.36.1).

IMMUNOHISTOCHEMICAL STAINS

The tumor cells were positive for CD20 but negative for CD3 and bcl-2. Further studies revealed that >99% of tumor cells were positive for Ki-67, and most of them were also positive for c-myc protein. Approximately 60% of tumor cells were CD10 positive and 30% were bcl-6 positive.

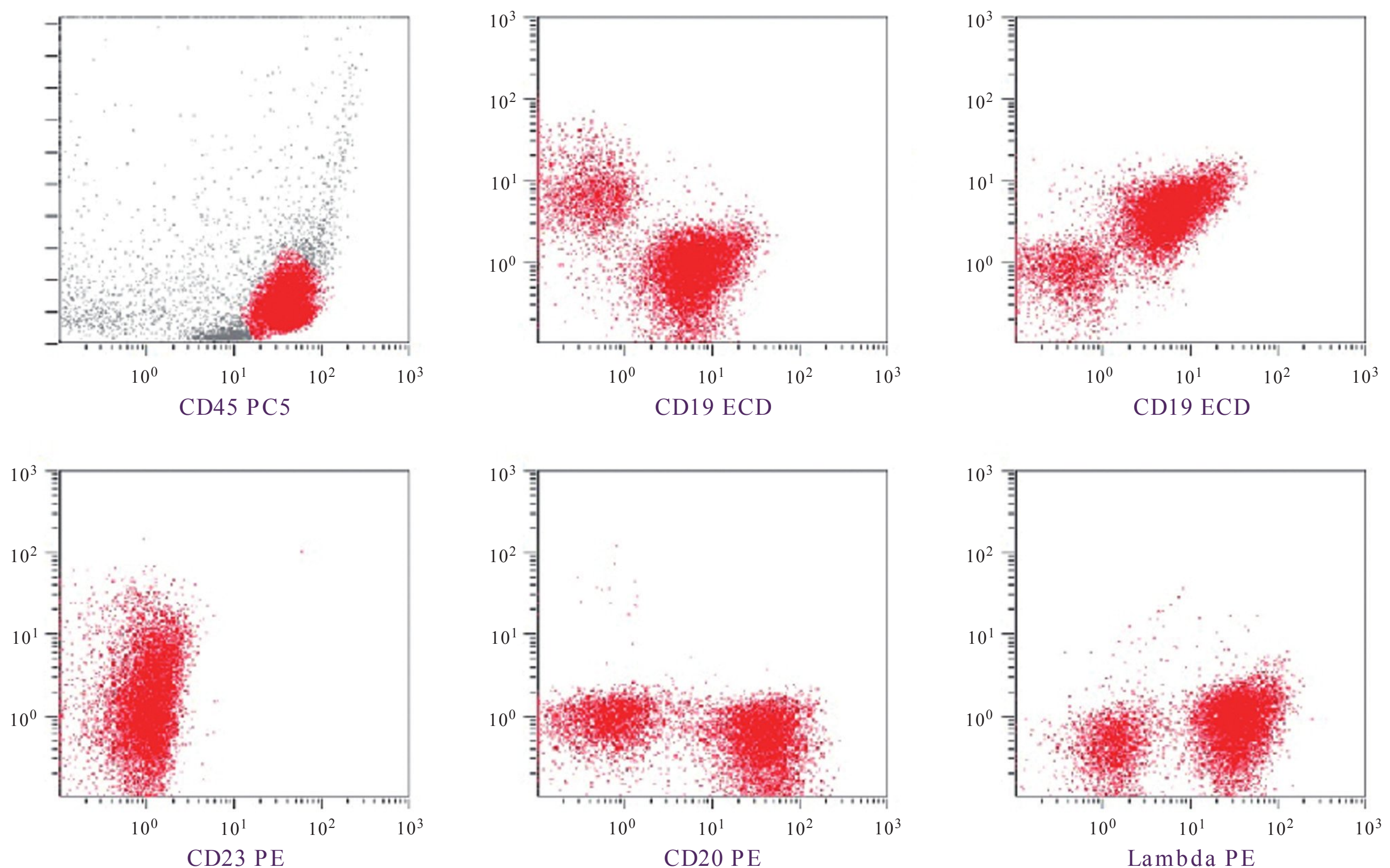


FIGURE 6.36.1 Flow cytometric analysis shows positive CD19, CD20, CD10, and FMC-7 in a monoclonal B population. The negative bcl-2 reaction helps to distinguish Burkitt lymphoma from follicular lymphoma and diffuse large B-cell lymphoma. SS, side scatter; PC5, phycoerythrin-cyanin 5; ECD, phycoerythrin-Texas Red; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

MOLECULAR GENETIC FINDINGS

A polymerase chain reaction (PCR) was performed on the paraffin section and showed a MYC oncogene rearrangement.

DISCUSSION

In 1958, Burkitt first discovered this tumor in Africa (1). He characterized this tumor as a sarcoma involving the jaws of African children. Subsequently, many cases of African lymphoma were reported outside the African continent and caused much confusion and controversy. In 1967, the World Health Organization (WHO) formed a committee of experts to investigate this tumor, and the definition of Burkitt lymphoma (BL) was then established (2). In the Working Formulation, BL falls under the category of small noncleaved cell lymphoma, as the tumor cells resemble the small noncleaved cells in the normal germinal centers of lymphoid follicles (3). However, these tumor cells are actually of intermediate size, between the sizes of large cell lymphoma and small lymphocytic lymphoma. Because BL occurs in a certain endemic area in Africa but is sporadic outside Africa, this tumor is also divided into endemic

and sporadic type. In the Revised European American Lymphoma (REAL) classification, this tumor is classified as BL (4). The 2001 WHO classification of lymphoid malignancies subdivides BL into endemic, sporadic, and immunodeficiency-associated subtypes (Table 6.36.1) (5–7). The originally proposed atypical BL subtype has changed back to Burkitt-like lymphoma (BLL), as that in the REAL classification. In the 2008 WHO classification, a new entity of B-cell lymphoma, unclassifiable, with features between diffuse large B-cell lymphoma (DLBCL) and BL was created (8,9). The new entity encompasses some of the BLL cases.

Morphology

The morphologic features in the endemic, sporadic, and immunodeficiency-associated subtypes are indistinguishable (10). An earlier report described the observation of an apparent transition from reactive follicles to follicular and diffuse BL in the sporadic form, but not in the endemic form (11). This difference, however, is not recognized in current studies.

The tumor cells are of medium size, approximately the same as those of the tingible-body macrophages invariably present in BL (Table 6.36.2). In tissue sections, the nuclear chromatin appears coarsely clumped with two to four small nucleoli and a thin rim of cytoplasm. The

TABLE 6.36.1

Comparison of Endemic, Sporadic, and Immunodeficiency HIV-Associated BL

	Endemic subtype	Sporadic subtype	HIV-associated
Annual incidence per 100,000 population	2.3–3.8	0.1–0.3	Variable
Age group with high incidence	4–8 years	Bimodal	HIV population
Male/female ratio	2.1:3.1	2.31:3.71	Male predominance
Initial presentation	Jaw lesion	Abdominal tumor	Lymphadenopathy
Bone marrow involvement	About 8%	16%–20%	Frequent
Central nervous system involvement	About 30%	5%–20%	Frequent
Leukemic form	Absent	Present	Present
Positive serologic test for EBV	88%–97%	20%	30–40%
EBV receptor on tumor cells	Common	Rare	Rare
EBV genome in tumor cells	100%	11%–20%	20%–40%
MYC gene breakpoint	Upstream of MYC	Within MYC	Within MYC
IG gene breakpoint	Joining region	Switch region	Switch region

BL, Burkitt lymphoma; EBV, Epstein–Barr virus.

histologic features show monotonous cohesive sheets of tumor cells with multiple mitotic figures and apoptotic tumor cells. The apoptotic bodies are frequently ingested by phagocytic cells, which are commonly referred to as tingible-body macrophages. The scattered tingible-body macrophages impart the so-called starry sky pattern, which is characteristic of but not pathognomonic for BL (Fig. 6.36.2). This feature can also be seen in lymphoblastic lymphoma, immunoblastic lymphoma, and occasionally other non-Hodgkin lymphomas. Therefore, using a touch preparation to identify the cytologic features of the tumor cells is particularly helpful for the diagnosis.

In touch preparations or bone marrow aspirates, the tumor cells are characterized by the moderate amount

of deep blue cytoplasm containing multiple cytoplasmic vacuoles (Figs. 6.36.3 and 6.36.4). The cytoplasm stains for methyl green pyronin (Fig. 6.36.5) due to its high content of polyribosome, whereas the lipid contents of cytoplasmic vacuoles are positive for Oil Red O (Fig. 6.36.6). The vacuoles are negative for periodic acid-Schiff (PAS), indicating the absence of glycoprotein or glycogen. The nuclei are regular, round, or oval with distinct nuclear membrane and more dispersed chromatin pattern than what is perceived in tissue sections (12).

Lymphomas with a morphology between BL and DLBCL are called non-BL in the Working Formulation (3) and BLL in the REAL (4) and 2001 WHO (5,6) classifications. To identify BLL and distinguish it from DLBCL is

TABLE 6.36.2

Characteristic Morphologic Features in Burkitt Lymphoma

Histologic pattern	Monotonous cohesive sheets of tumor cells with multiple mitotic figures and scattered tingible-body macrophages (starry sky pattern)
Cytology	Medium-sized cells with immature chromatin pattern and multiple small nucleoli; blue, vacuolated cytoplasm on Wright–Giemsa–stained preparations
Specific features	Starry sky pattern with characteristic cytology on touch preparations

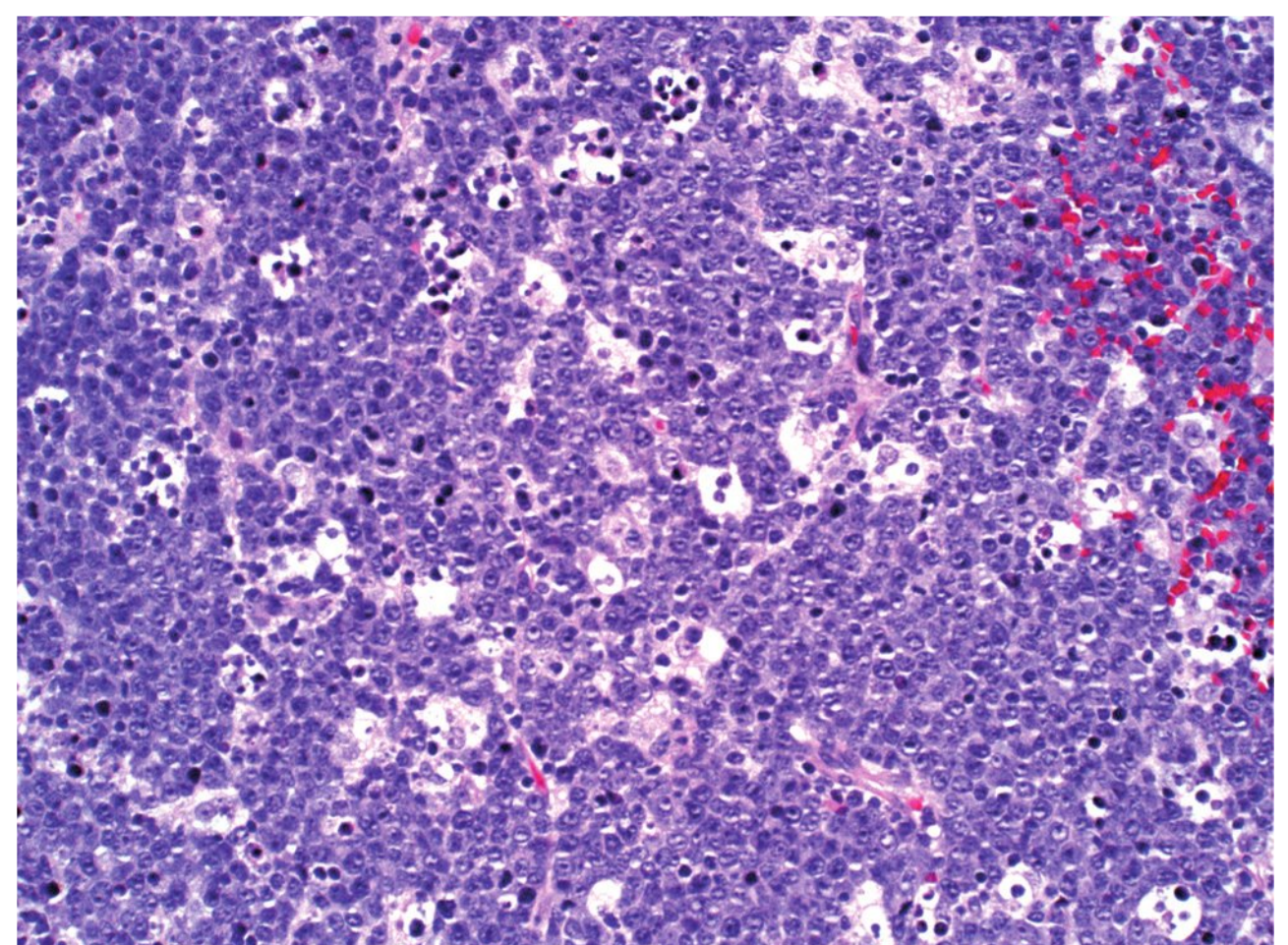


FIGURE 6.36.2 Histology of a lymph node biopsy is characterized by the prominent starry sky pattern due to the presence of multiple tingible-body macrophages. Hematoxylin and eosin, 20 × magnification.

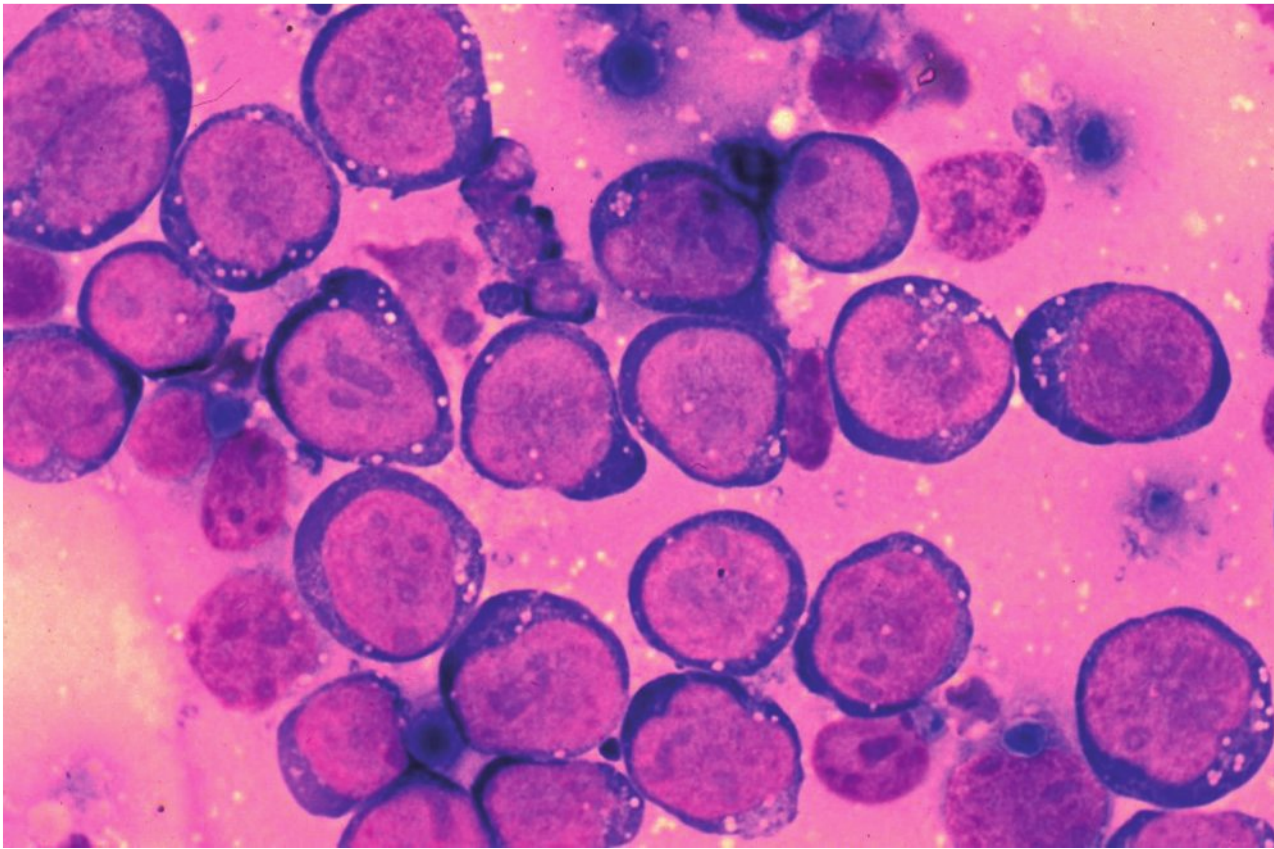


FIGURE 6.36.3 Touch imprint from a lymph node reveals the characteristic cytology of deep basophilic cytoplasm with vacuolization, and immature chromatin pattern with inconspicuous multiple nucleoli. Wright–Giemsa, 150× magnification.

important clinically because the treatments of these two tumors are quite different. The major morphologic differences between BL and BLL are the presence of pleomorphic nuclei in BLL that are irregular in configuration and vary in size, shape, and number of nuclei (varying from one to three) in each cell (Fig. 6.36.7). The nucleoli in BLL cells are usually more prominent than BL cells and are frequently eosinophilic (10). One study found that the most distinguished morphologic feature in BLL is the presence of a single prominent nucleolus (13) (Fig. 6.36.8). Another study showed that the pleomorphic features in BLL are also due to the existence of various cell populations (14). In the 19 cases studied, the tumor cells were composed of at least 10% of Burkitt cells and various proportions of centroblasts and immunoblasts with plasmacytic differentiation. BL with plasmacytoid differentiation is classified as

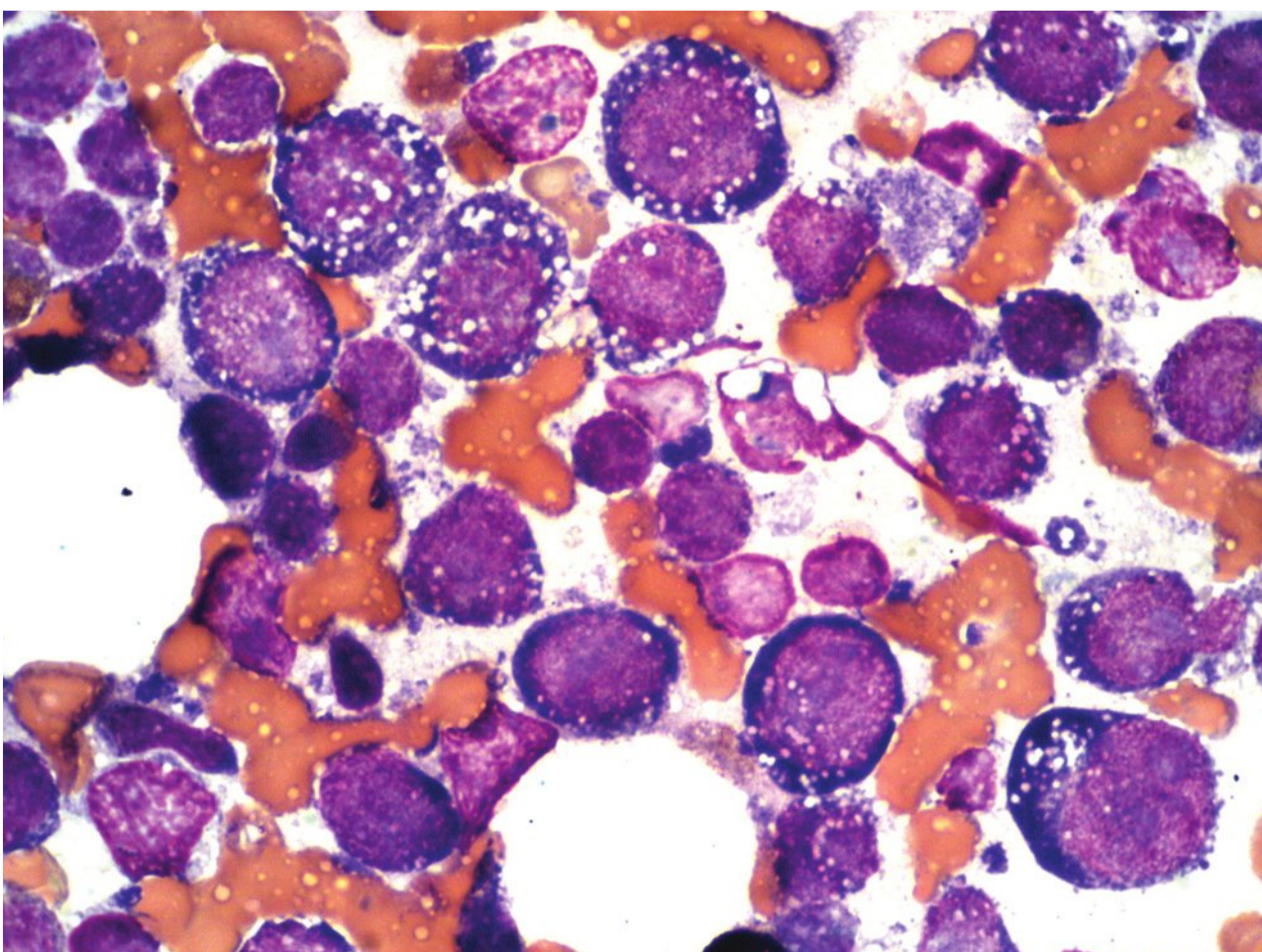


FIGURE 6.36.4 Bone marrow aspirate shows a cluster of Burkitt lymphoma cells with the same characteristics as those seen in the lymph node imprint. Wright–Giemsa, 100× magnification.

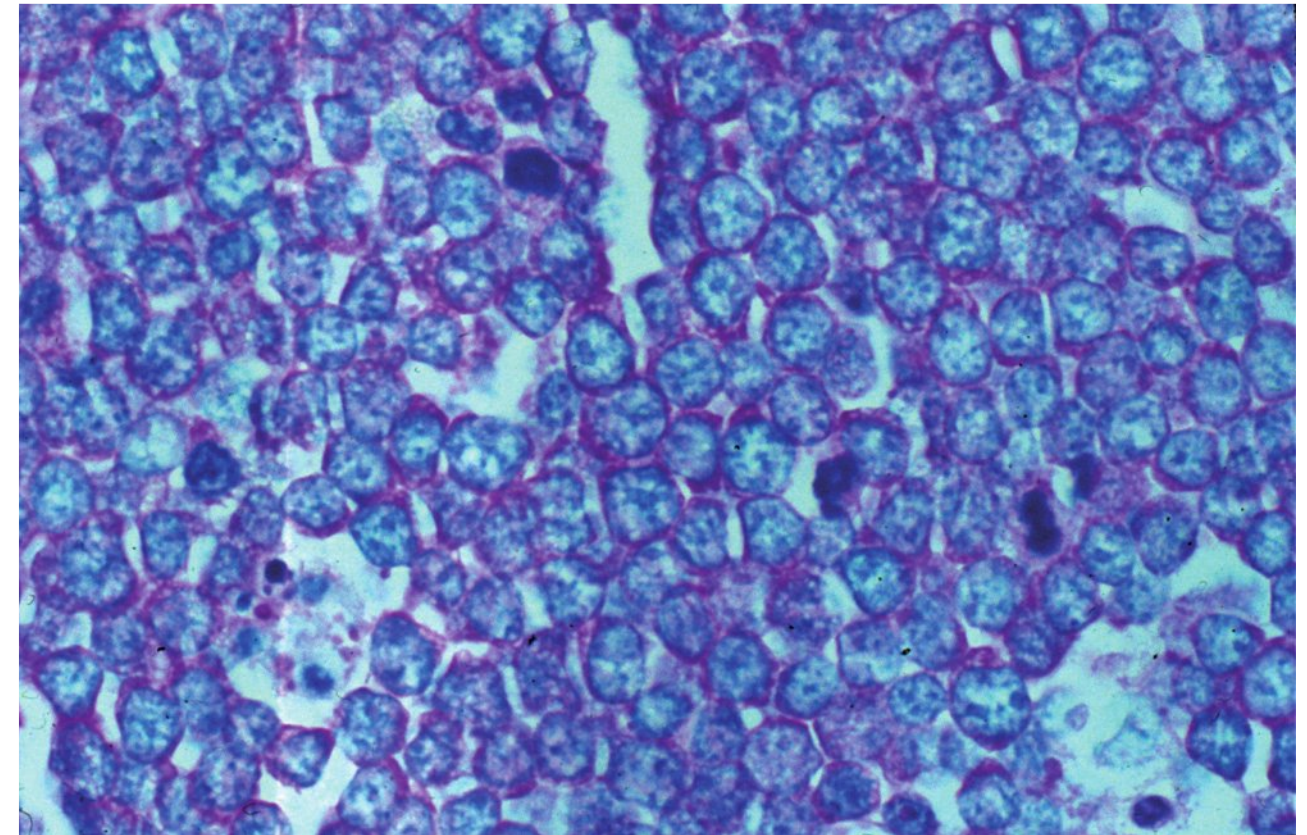


FIGURE 6.36.5 Lymph node biopsy shows positive methyl green pyronin stain in tumor cells. 60× magnification.

a variant of BL and is separated from BLL in the 2001 WHO classification (5,8). In spite of the morphologic deviation, many of the BLL cases share the same molecular expression profile as the classical BL cases and are considered true BL (8).

The new entity of DLBCL/BL defines a heterogeneous group of lymphomas, which shows not only overlapping morphology between DLBCL and BL, but also distinct immunophenotype and molecular genetic characteristics (Table 6.36.3) (9,15). This group of lymphomas may show variable nuclear size and shape that are deviated from typical BL cells, or the nuclear size is intermediate between DLBCL and BL (Figs. 6.36.9–6.36.13). In rare cases, the small nuclei and finely granular chromatin resemble those of lymphoblastic lymphoma. Cases with BL morphology but atypical immunophenotype and/or molecular genetic features should be considered in this category. However, if a case shows morphologic and immunologic features typical for BL, but no MYC gene rearrangement, it should not be classified as DLBCL/BL. Conversely, cases showing typical morphology and immunophenotype of DLBCL, even with MYC gene rearrangement, should be diagnosed as DLBCL.

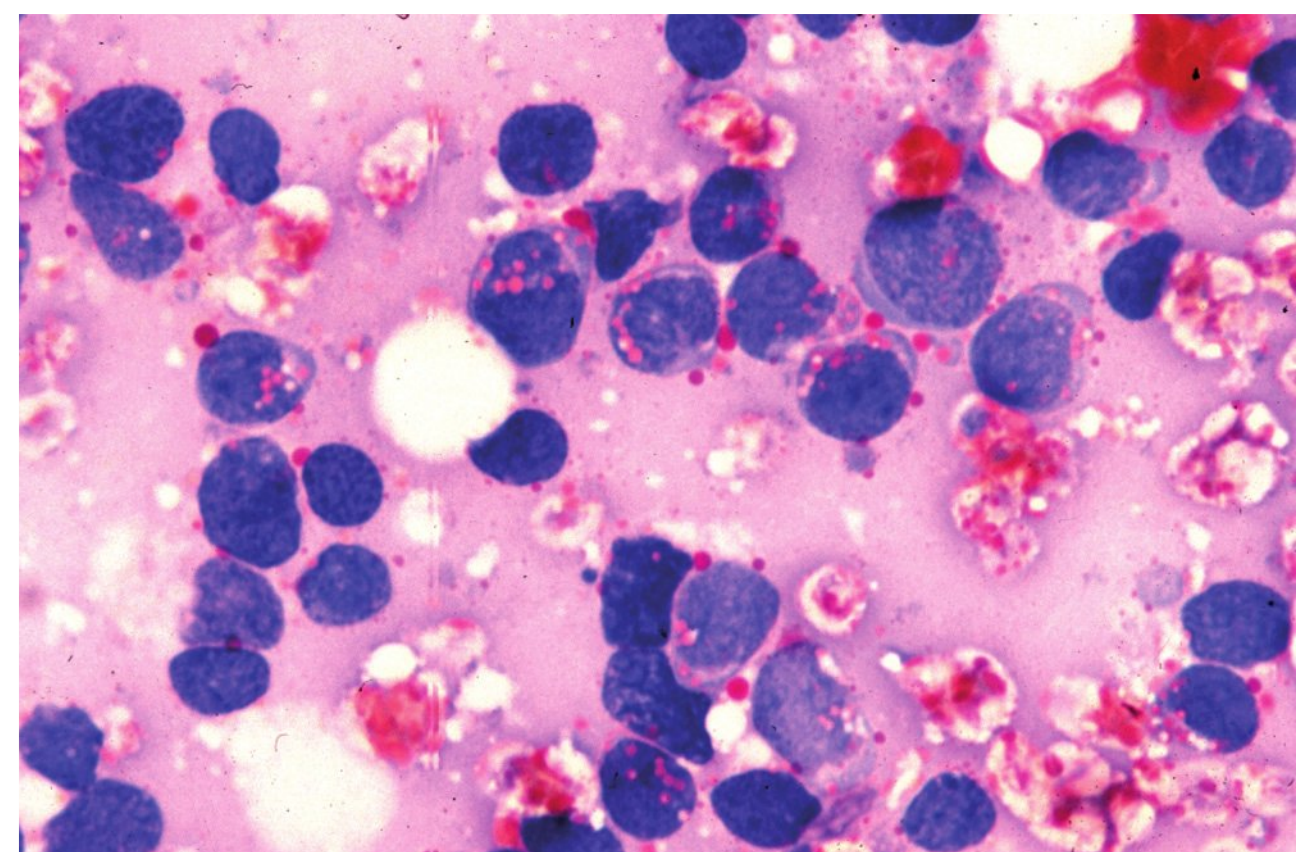


FIGURE 6.36.6 Lymph node imprint reveals positive Oil Red O stain of the cytoplasmic vacuoles of tumor cells. 100× magnification.

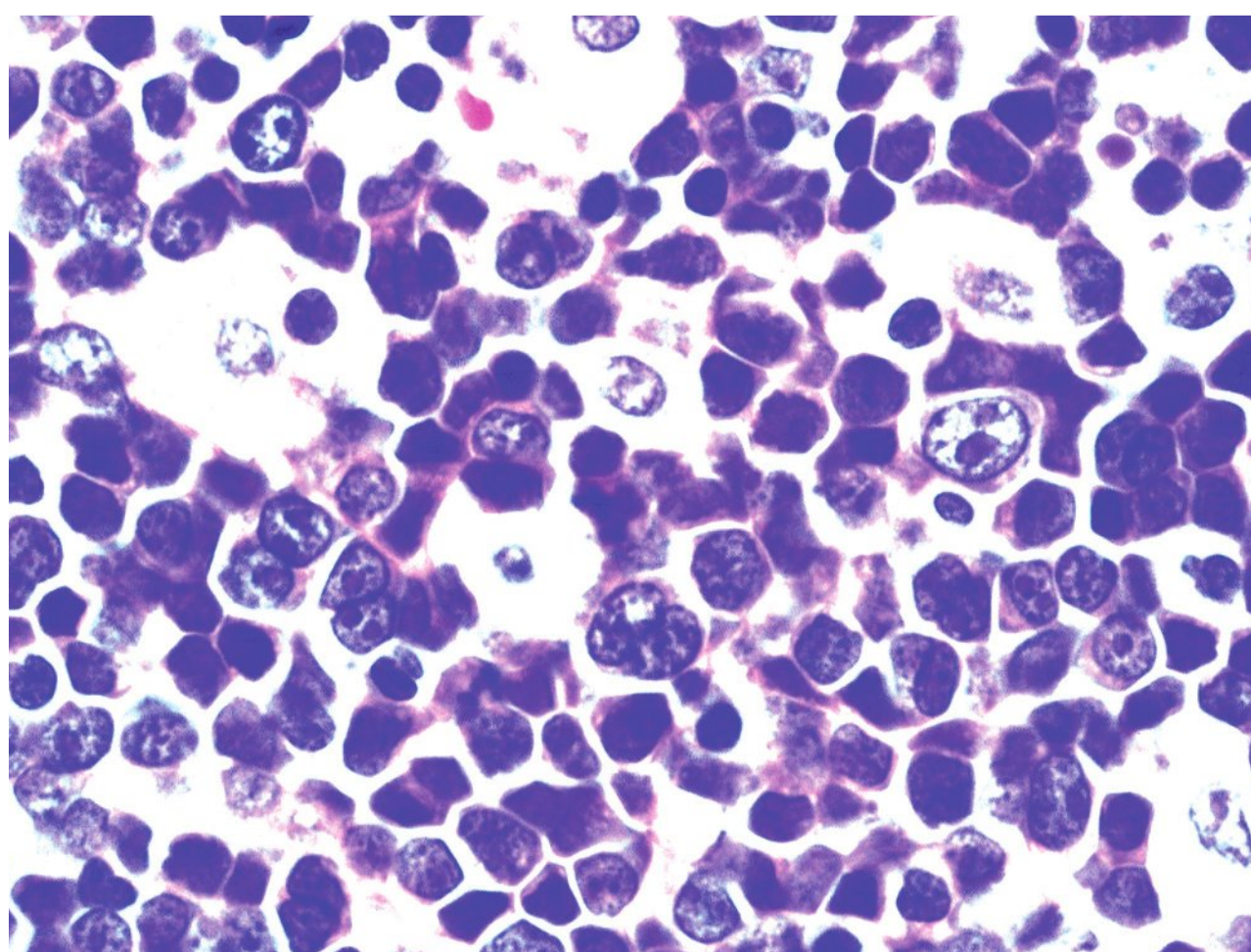


FIGURE 6.36.7 Lymph node biopsy from a case of BLL shows pleomorphic morphology with a few multinucleated cells. Note the tingible-body macrophages. Hematoxylin and eosin, 100× magnification.

Immunophenotype

BL is a tumor of follicular center cell origin. Therefore, the immunophenotype of BL is similar to that of follicular lymphoma. Besides B-cell markers (i.e., CD19, CD20, CD79a, and monoclonal surface immunoglobulin [Ig]), BL also expresses other markers that are specific for follicular lymphoma (i.e., CD10 and bcl-6) (5,12). However, bcl-2 is negative for BL, so this marker may help to distinguish these two tumors (5,16). Recently, a human germinal center-associated lymphoma gene was identified in gene expression profiling (GEP) studies of DLBCL (17). Its protein product is expressed in all germinal center-derived lymphomas, including BL. B-lineage-associated transcription

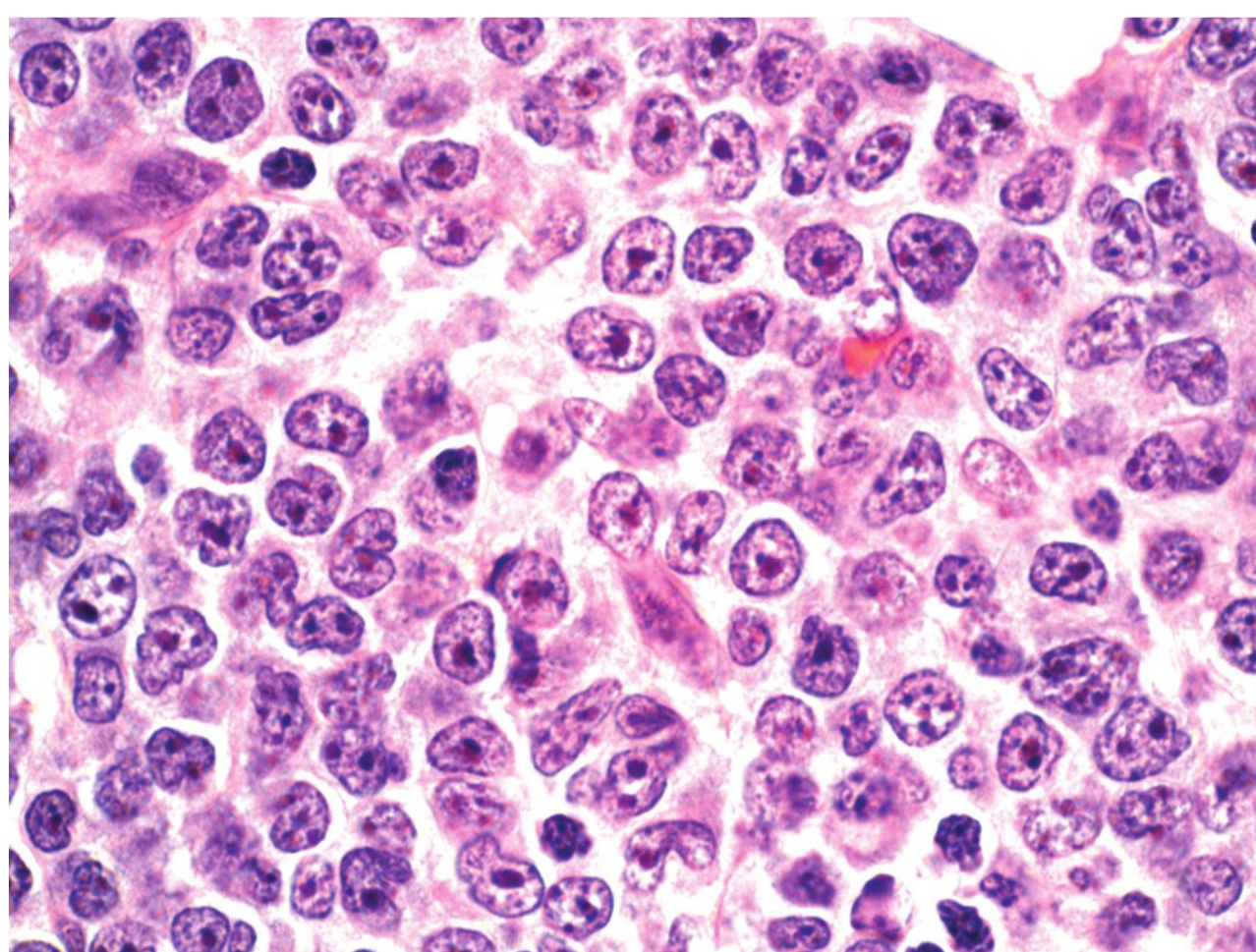


FIGURE 6.36.8 Bone marrow aspiration cell block shows sheets of tumor cells with a single nucleolus in most tumor cells. Cytogenetic studies demonstrated a complex karyotype with t(8;14) and t(14;18) translocation consistent with DLBCL/BL. Hematoxylin and eosin, 100× magnification.

factors, namely PAX5, BOB.1, and PU.1, are also expressed on BL cells in a current study (18).

The germinal center B-cell-like subgroup of DLBCL, particularly those transformed from follicular lymphoma, may also share the same immunophenotype with follicular lymphoma (16,19). These cases are especially difficult to distinguish from BL. In the 2001 WHO classification, a high proliferation fraction (Ki-67 > 99%) is the only acceptable immunologic marker for the diagnosis of BL without cytogenetic studies (5) (Fig. 6.36.14). However, some DLBCL may also have a very high percentage of Ki-67. The current consensus is that Ki-67 percentage is an important factor in differential diagnosis, but this factor alone does not rule out or rule in a certain diagnosis. A few markers may provide further support to distinct these two entities, such as positive c-myc protein in BL (16) and positive bcl-2 and IRF4/MUM1 proteins, in DLBCL. A recent study demonstrated that CD44, a lymphocyte homing receptor, is strongly positive in CD10+ DLBCL, but is negative in BL (20). An immunophenotype of CD44−/CD38+/TCL-1+ may predict the presence of MYC rearrangement in BL, but it is not present in MYC-negative DLBCL (21).

The reactivities of other B-cell markers included 28% for CD21, 66% for CD22, and 75% for FMC-7 in one study (22). CD21 is the Epstein-Barr virus (EBV)/C3d receptor, so its expression depends on the EBV status of the tumor. Therefore, the endemic BL cases express CD21, but the majority of sporadic BL cases are CD21 negative (5).

In a comparison of 19 cases of BLL and 25 cases of BL in human immunodeficiency virus (HIV)-infected patients, BLL cases were found to have statistically higher percentages of activation antigens (CD39 and CD70) and of the CD11a/lymphocyte function-associated antigen-1 (LFA-1) adhesion molecule than the BL cases (14). Another study revealed that BL contains higher percentages of bcl-6 (Fig. 6.36.15) and c-myc (Fig. 6.36.16) but a lower percentage of bcl-2 protein than BLL does (23). These so-called BLL cases could well be DLBCL/BL cases in the new classification. The immunodeficiency-associated BL is also specific in the expression of CD45RO, (a T-cell marker) in tumor cells (24).

The sporadic subtype but not the endemic subtype of BL may have a leukemic phase, which is identical to the morphology of L3 type acute lymphoblastic leukemia. L3 can be distinguished from L1 and L2 by the presence of surface Ig and the absence of terminal deoxynucleotidyl transferase (TdT) and CD34 (5). However, the 2008 WHO classification discourages the usage of the term L3 acute lymphoblastic leukemia to describe Burkitt leukemia.

Four cases of de novo CD5-positive BL/leukemia have been reported (25). These cases showed dual CD5/CD19 or CD5/CD20 with positive FMC-7 and negative CD23 and CD10; their immunophenotype mimics that of mantle cell lymphoma. The folded nuclei in the blasts are another feature similar to the blastoid form of mantle cell lymphoma. In these cases, only molecular genetics can accurately identify the tumor as BL and exclude mantle cell lymphoma.

TABLE 6.36.3			
Comparison of BL, DLBCL/BL, and DLBCL			
	BL	DLBCL/ BL	DLBCL
Cell size	Medium	Between BL and DLBCL	Large
Nucleoli	Multiple, small	Between BL and DLBCL	Prominent
Mitotic rate	High	High	Low
Starry sky pattern	Common	Common	Less common
Proliferation fraction	>90%	>90%	<90%
bcl-2 protein	Negative	Positive	Positive
IRF4/MUM1	Negative	Negative	Positive
MYC-IG gene	Common	Some cases	Rare
MYC-non-IG gene	No	Some cases	Rare
BCL-2 rear alone	No	Rare	Some cases
BCL-6 rear alone	No	Rare	Some cases
Karyotype	Simple	Complex	Complex

BL, Burkitt lymphoma; DLBCL/DL, B-cell lymphoma, unclassifiable, with features between DLBCL and BL; DLBCL, diffuse large B-cell lymphoma; rear, rearrangement

Comparison of Flow Cytometry and Immunohistochemistry

For the diagnosis of BL, immunohistochemistry is superior to flow cytometry because flow cytometry can only demonstrate CD10 for a specific diagnosis. Additional markers, such as bcl-2, bcl-6, c-myc, and Ki-67, can be demonstrated by immunohistochemistry and make the diagnosis more reliable.

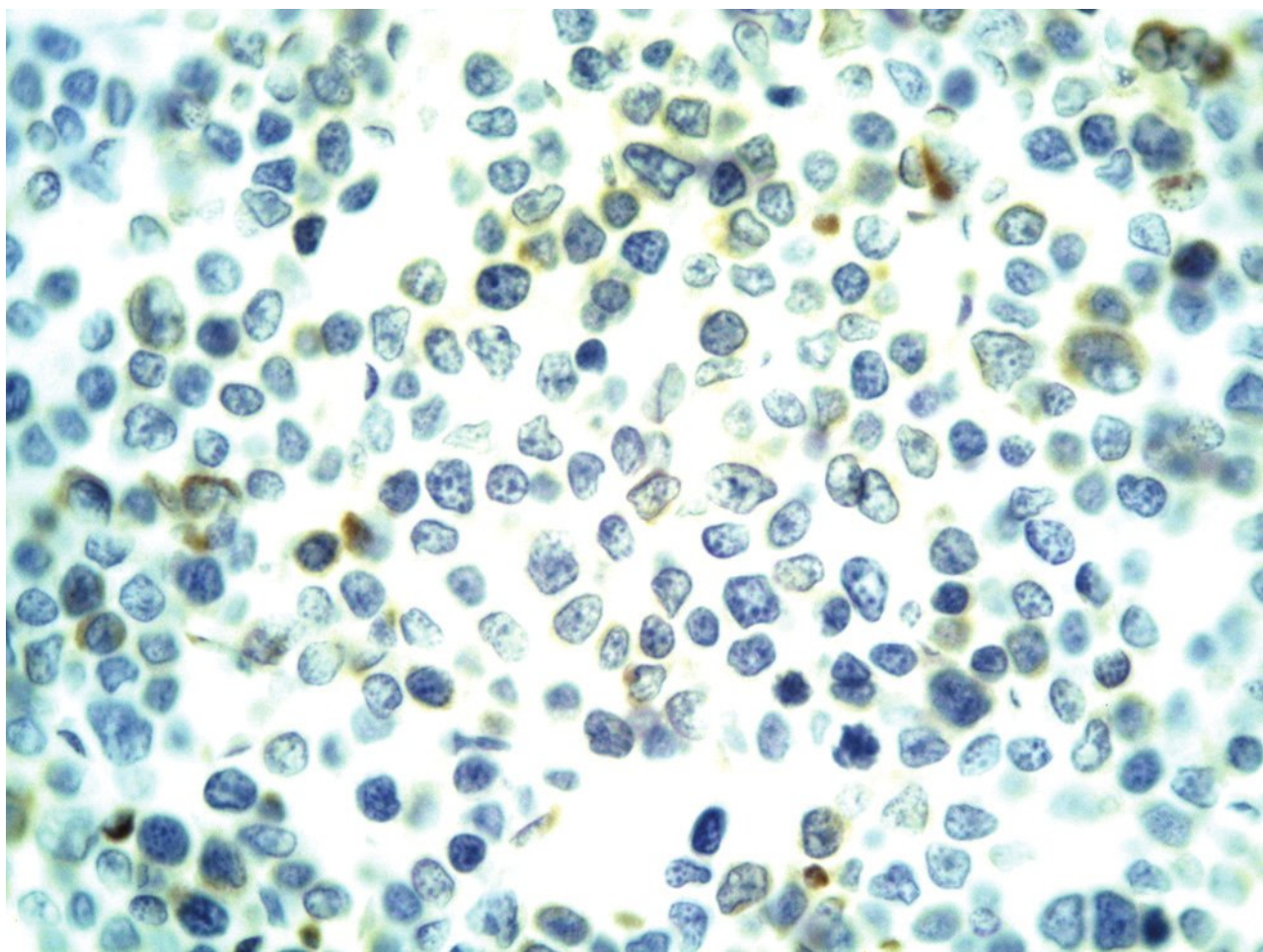


FIGURE 6.36.9 Lymph node section from an autopsy case shows pleomorphic tumor cells with negative bcl-2 stain. Antemortem cytogenetic study of bone marrow shows t(8;22) translocation. Immunophenotype is composed of CD10+, bcl-2–, bcl-6+, Ki-67 >90%, consistent with DLBCL/BL. Immunoperoxidase, 60× magnification.

Molecular Genetics

In the 2001 WHO classification, rearrangement or translocation of the MYC oncogene is the prerequisite for the diagnosis of BL (5). However, this is no longer the basic requirement in the 2008 WHO classification (8). In 80% of BL cases, the abnormality is t(8;14)(q24;q32) (Fig. 6.36.17), representing the translocation of the MYC oncogene on chromosome 8 to juxtaposing the heavy-chain gene on chromosome 14 (26). The remaining cases involve the k (15%) or l (5%) light-chain gene, showing t(2;8) (p12;q24)

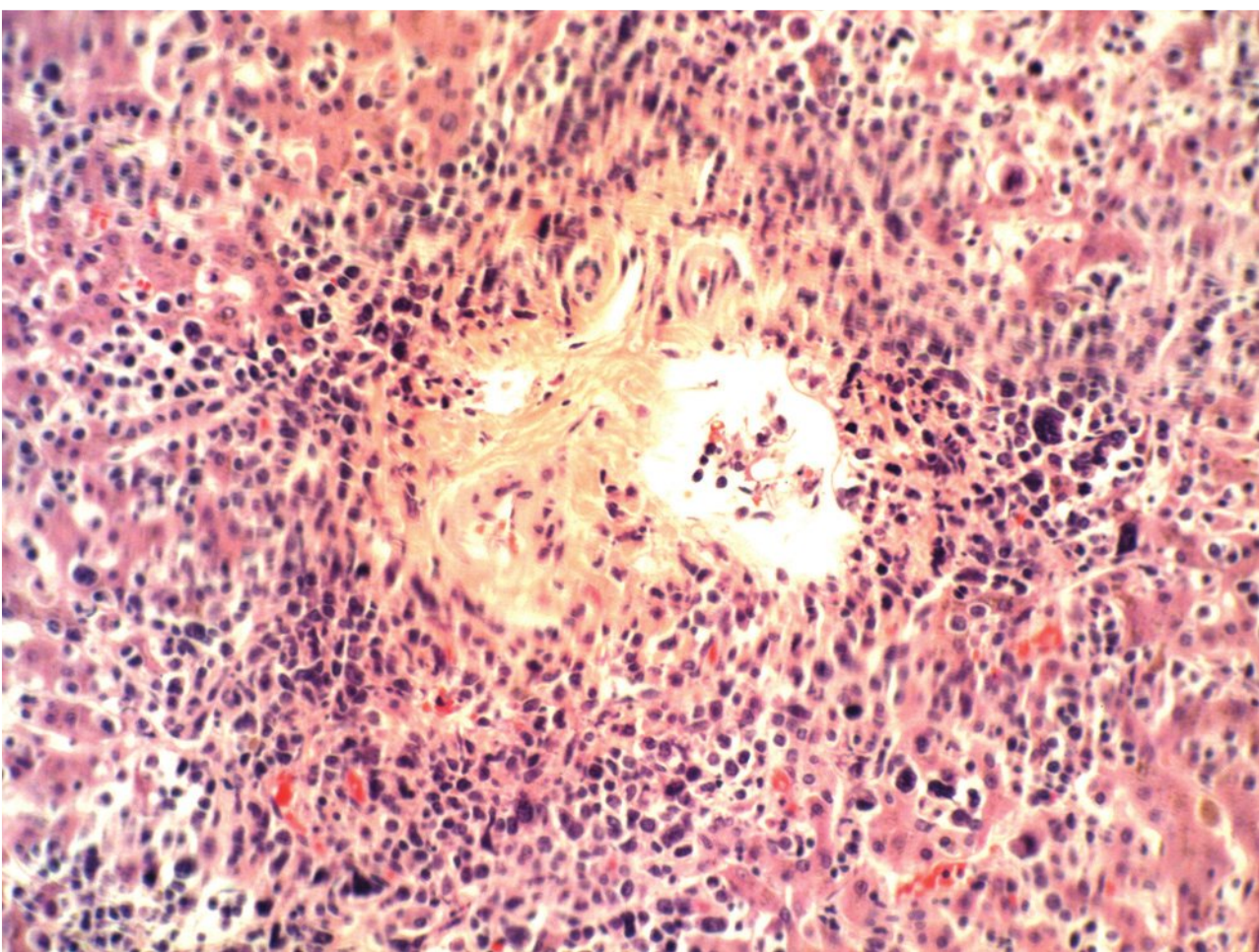


FIGURE 6.36.10 Liver section from the same case as Figure 6.36.9 shows tumor cell infiltration in the portal area spreading into adjacent liver sinuses. Hematoxylin and eosin, 10× magnification.

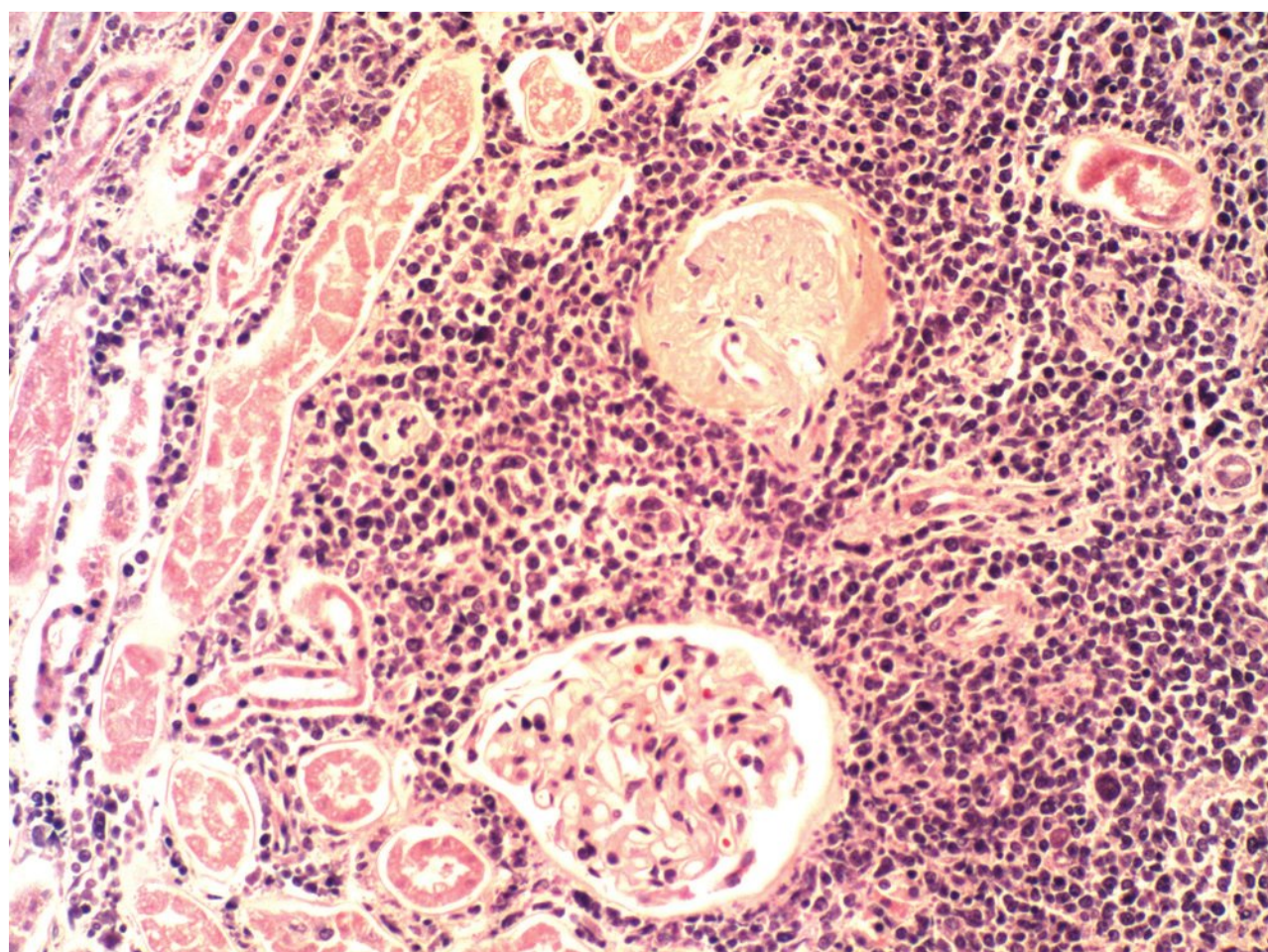


FIGURE 6.36.11 Kidney section from the same case as Figure 6.36.9 shows tumor cell infiltration in the interstitial tissue between the glomeruli and renal tubules. Hematoxylin and eosin, 20× magnification.

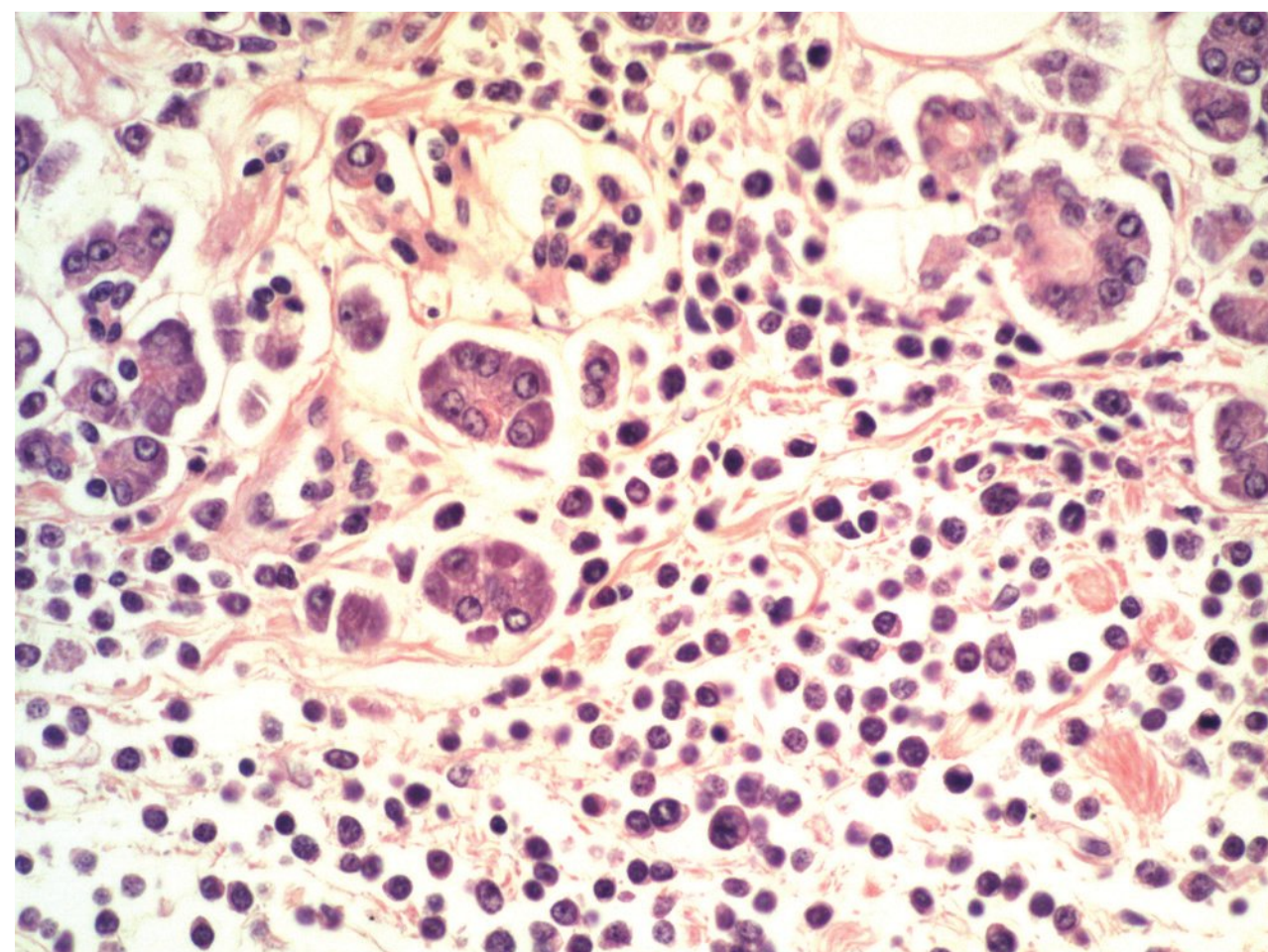


FIGURE 6.36.13 Pancreatic section from the same case as Figure 6.36.9 shows interstitial tumor cell infiltration. Hematoxylin and eosin, 40× magnification.

or $t(8;22)(q24;q11)$ (Fig. 6.36.18), respectively. When the partner gene of MYC is a nonimmunoglobulin gene, the tumor is more likely to be DLBCL/BL or DLBCL. These cytogenetic abnormalities can now be detected not only by karyotyping but also by Southern blotting, PCR, and fluorescence in situ hybridization (FISH) (27). Cryptic IgH-MYC rearrangement may require three-color, dual fusion FISH probe set for its detection (28).

This translocation results in deregulation of the MYC gene, which leads the tumor cells remaining constantly in the cell cycle (29). However, MYC gene translocation can also be seen in other lymphomas and/or leukemias, such as DLBCL, transformed follicular lymphoma, blastoid mantle cell lymphoma, plasma cell myeloma, lymphoblastic lymphoma, Richter transformation, and prolymphocytoid

transformation from chronic lymphocytic leukemia (30–35). In contrast, up to 10% of BL cases may lack a detectable MYC translocation by FISH (8). A recent study found that even in cases without MYC translocation, the MYC gene is still upregulated to the level comparable to those with MYC translocation (36). The same study also showed that overexpression of MYC is associated with downregulation of a group of micro-RNA that targets MYC gene. This could be an alternative mechanism for lymphomagenesis in cases lacking MYC translocation (36). A specific MYC-related miRNA profile has been identified in cases of BL (37).

Although endemic and sporadic BL and BLL all carry the same cytogenetic translocation (29), the breakpoints are different in various tumors (11). In sporadic and

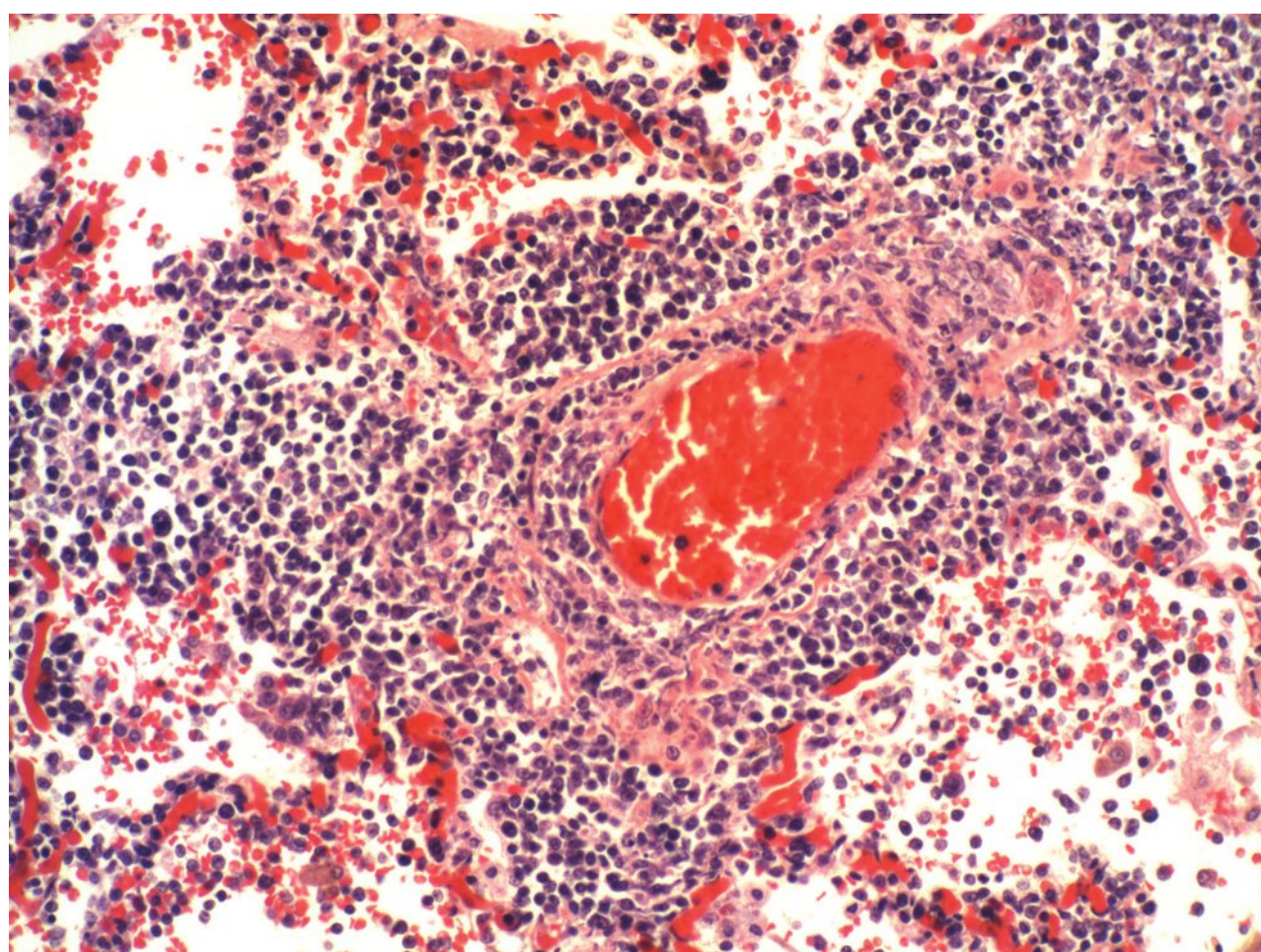


FIGURE 6.36.12 Lung section from the same case as Figure 6.36.9 shows perivascular and intra-alveolar tumor cell infiltration. Hematoxylin and eosin, 20× magnification.

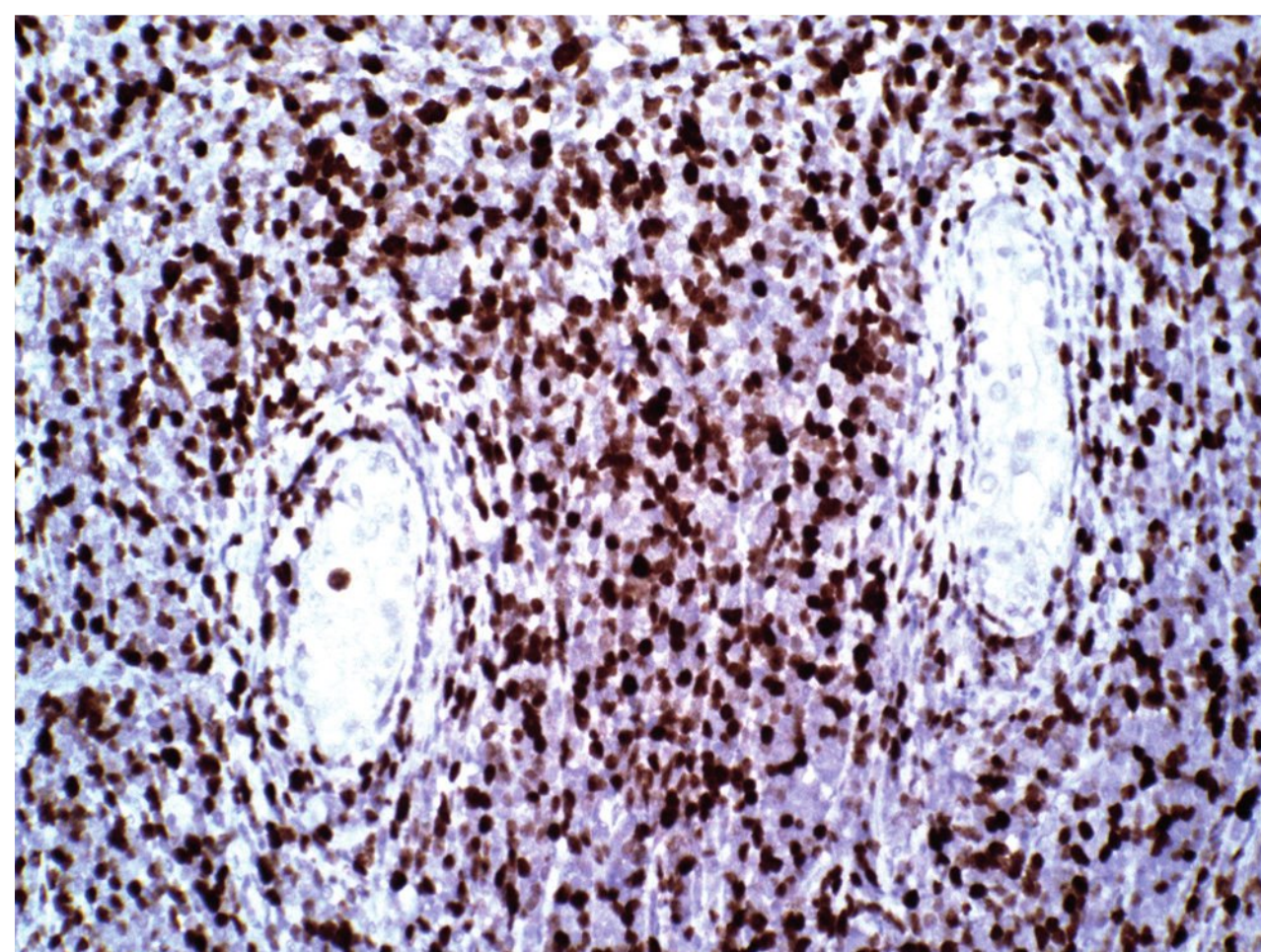


FIGURE 6.36.14 Testicular Burkitt lymphoma shows 100% proliferation fraction as demonstrated by Ki-67 staining. Immunoperoxidase, 20× magnification.

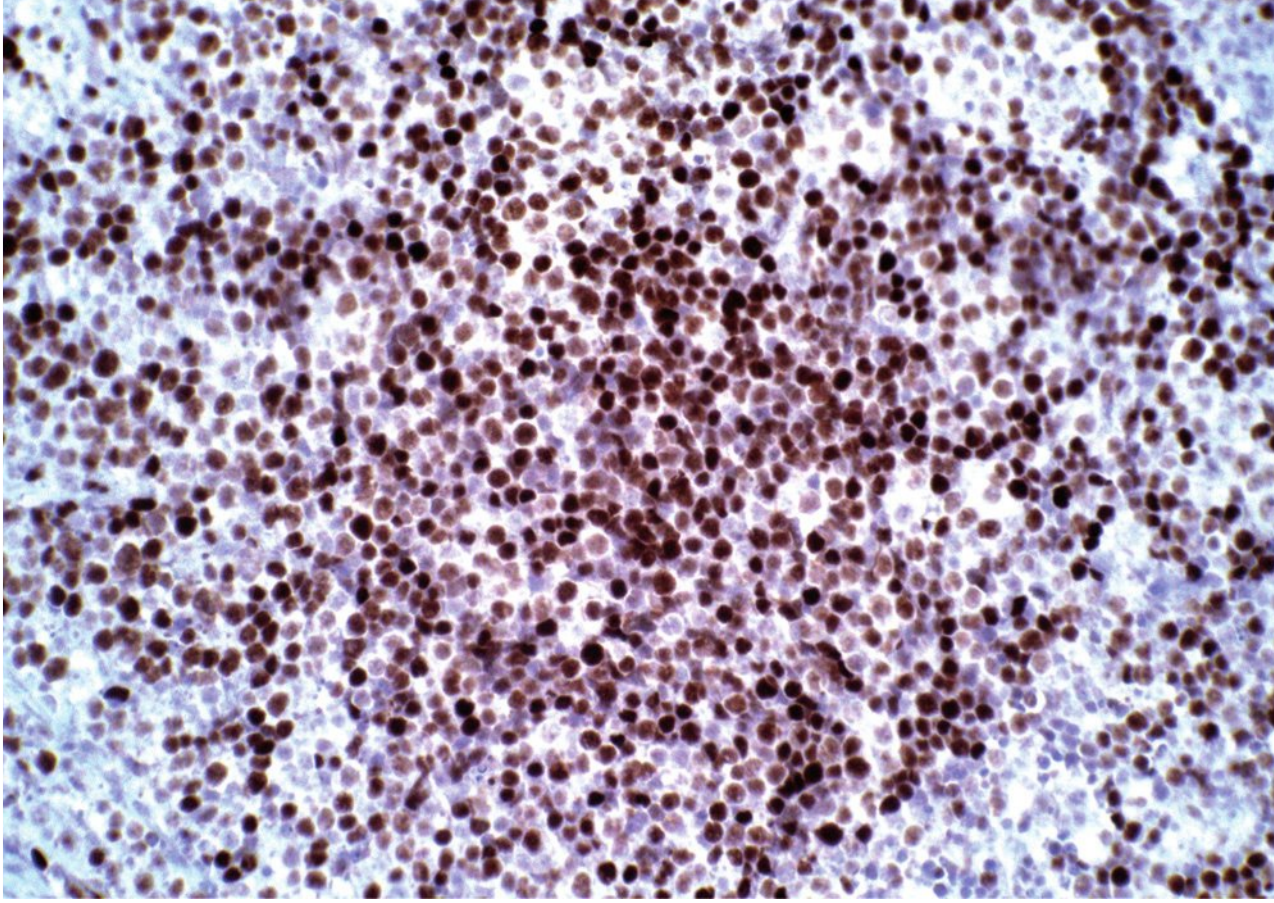


FIGURE 6.36.15 Lymph node biopsy shows bcl-6–positive tumor cells. Immunoperoxidase, 20× magnification.

immunodeficiency-associated BL, the breakpoint in chromosome 8 is often within the MYC gene, whereas the breakpoint in 75% of endemic BL is upstream of the MYC gene (7,38). In contrast, the breakpoint on chromosome 14 involves the heavy-chain joining region in endemic BL, whereas sporadic and immunodeficiency-associated BL involves the heavy-chain switch region (7,39,40).

In South America, the predominant breakpoints in BL cells differ from those of sporadic and endemic BL cells (41). Furthermore, the breakpoints of the BLL cells may lie far from the MYC gene (42). In some studies, MYC rearrangement was demonstrated in only a small percentage (43) or in none (44) of the BLL cases studied.

One of the controversial issues was the detection of BCL-2 rearrangement in BL cases. Yano et al. (44) found that 3 of 11 BLLs showed BCL-2 rearrangement with comigration of the heavy-chain gene, indicating t(14;18).

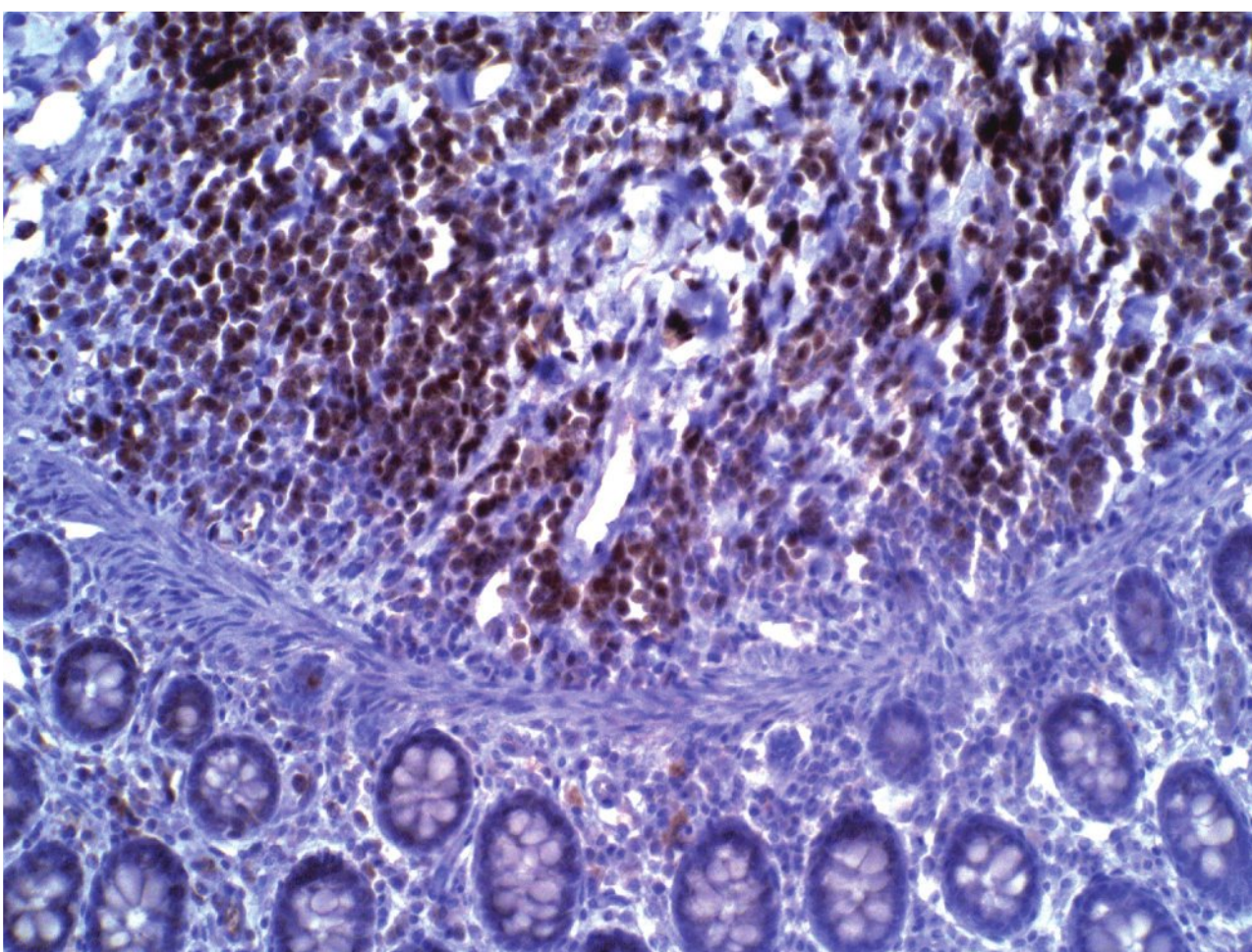


FIGURE 6.36.16 Colon biopsy reveals positive MYC protein staining in tumor cells in the submucosal region. Immunoperoxidase, 20× magnification.

The rearrangement of MYC was not detected in any of these cases. In another study, the BCL-2 gene was not demonstrated in 12 cases of acquired immunodeficiency-associated BL (14). However, another study found a high frequency of t(14;18) in Burkitt leukemia (L3) (45).

In the 2008 WHO classification, these cases may be classified as DLBCL/BL (9). Cases with both MYC and BCL-2 translocation (double-hit lymphoma) define an aggressive group of lymphomas (46). On the other hand, patients with simple karyotype but including both MYC and BCL-6 rearrangements are mostly BL cases with better survival as compared to those without BCL-6 rearrangement (47). Rarely cases may carry MYC, BCL-2, and BCL-6 rearrangements (triple-hit lymphoma) (9).

BL and BLL may also show p53 protein overexpression or p53 mutation (48,49). Inactivation of wild-type p53 function can render the BL cell line significantly more tumorigenic in athymic mice (50). The Rb2/p130 gene is also mutated in most cases of endemic BL but in fewer cases of sporadic BL (51).

In terms of distinction between BL and DLBCL, two recent studies with GEP showed that even cytogenetics was not entirely reliable. The former may or may not have c-myc but may have BCL-2 rearrangement, and the latter may have MYC gene rearrangement (52,53). However, they have distinctly different signatures in GEP.

A striking early discovery is the association of BL with EBV, a relationship that has now been found in many lymphoid tumors (14). EBV has been isolated from virtually all tumor samples from Africa. EBV receptor has also been found in most of the BL cell lines originating from Africa. In contrast, sporadic BLs do not usually carry EBV and EBV receptor, with a frequency of only 11% to 20% (26). EBV is identified in only 25% to 40% of immunodeficiency-associated cases (5). EBV antibodies are detected in 88% to 97% of patients in the endemic area but in only 20% of patients in the nonendemic area (54).

Conflicting results are demonstrated in studies of the association between EBV and BLL. One study showed that EBV DNA was detected by in situ hybridization and Southern blot techniques in 79% of BLL cases but in only 48% of sporadic BL cases (14). Another study failed to demonstrate EBV DNA by PCR in all 12 BLL cases (27). EBV is also more commonly seen in HIV-positive than HIV-negative BL cases (55). Because disrupted and aberrant expressions of the viral genome have recently been found in cases of sporadic BL in the United States that were interpreted as EBV-negative in standard screening, the high negative rate in sporadic BL or BLL may be due to the low sensitivity of the test (56).

Another caution is that the results obtained from BL-derived cell lines may differ from those of primary tumor tissue for molecular analysis of EBV genomes (57). A study of EBV-BL association in Taiwan found that EBV-encoded small RNAs were seen mostly in head and neck BL, but seldom in abdominal BL (58). This finding may partly explain the difference in EBV positivity between the endemic and sporadic BLs.

The etiologic role of EBV in BL, however, is controversial. The demonstration of monoclonality of EBV infection

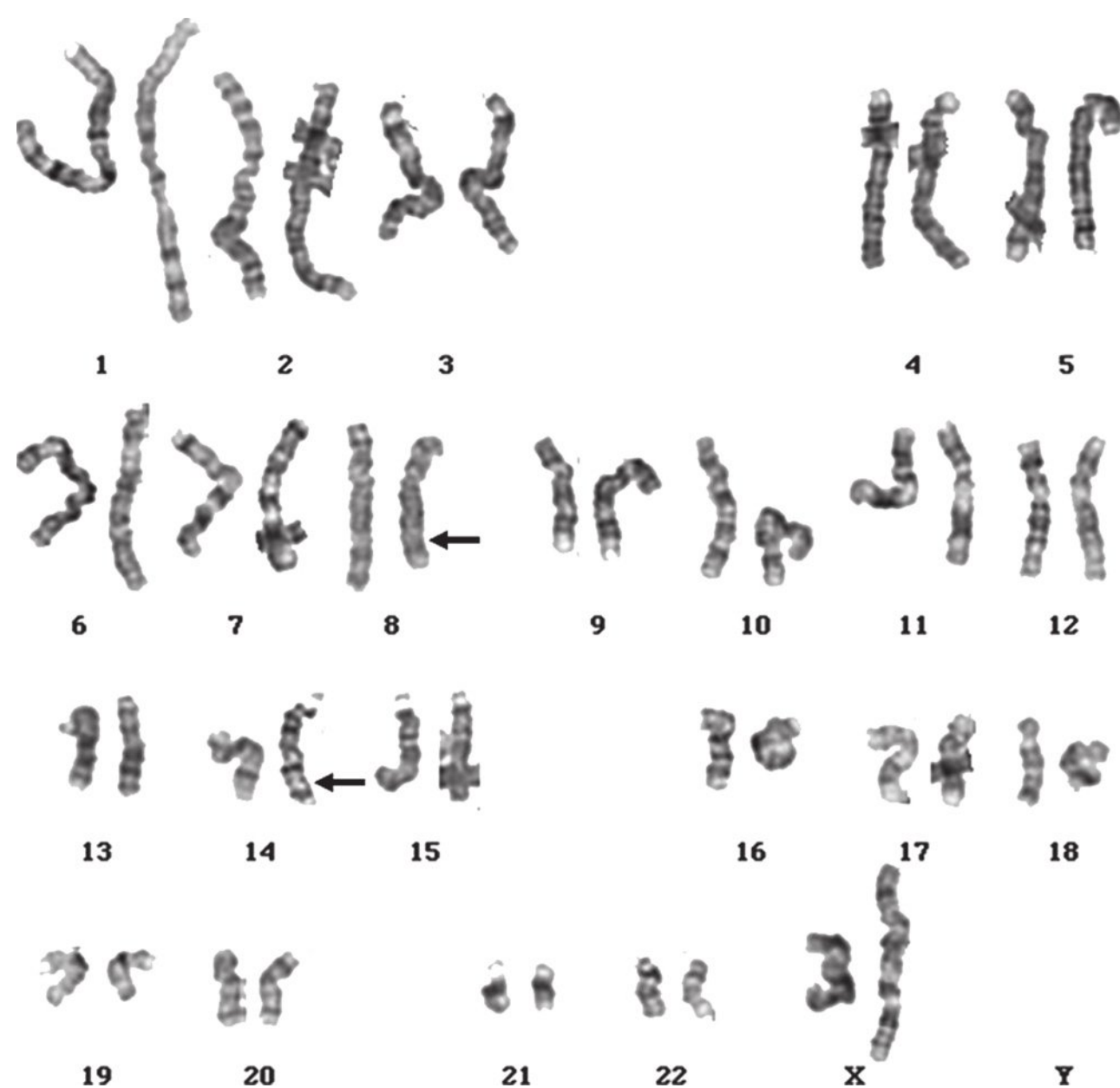


FIGURE 6.36.17 Cytogenetic study shows a complex karyotype with $t(8;14)(q24;q32)$ from a case of DLBCL/BL. (From Sun T, Atlas of Hematologic Neoplasms. Springer, 2009, with permission).

in BL by molecular analysis of EBV terminal repeats supports this assertion (59). However, because BL cells failed to express the EBV-encoded antigens LMP-1 (latent membrane protein-1) and EBNA-2 (EBV nuclear antigen-2) in tumor cells in several studies (60,61), the etiologic role of EBV is challenged. In fact, the predisposing factors of BL may be multiple, including bacterial, viral (EBV, HIV,

mosquito borne arboviruses), and parasitic (malaria), leading to defective T-cell regulation of EBV-infected B cells (5,7).

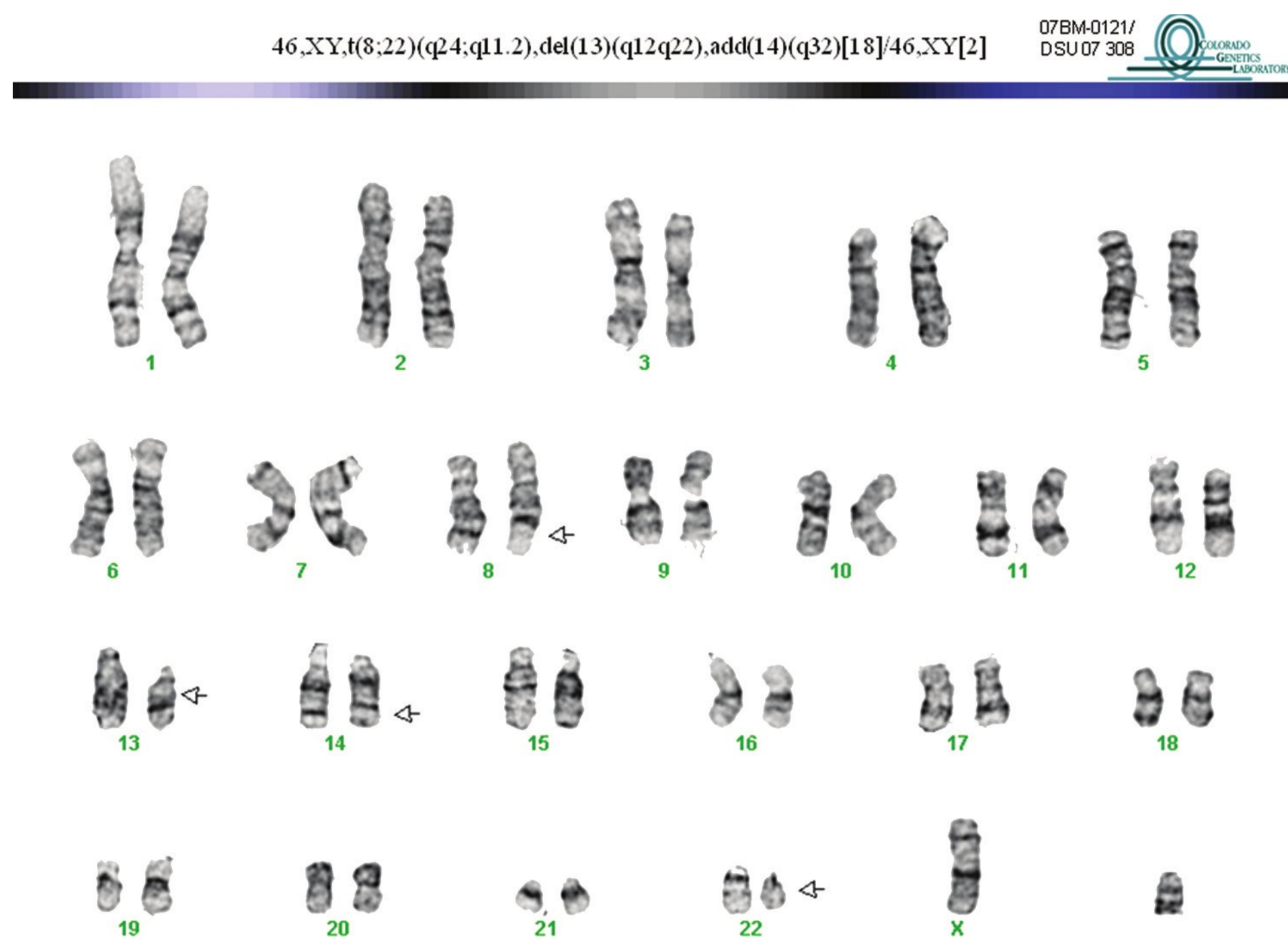
The current patient is a typical case of sporadic BL showing an ileocecal location and a rapidly progressive clinical course. The biopsy of the cecal mass showed characteristic features of Burkitt cells, and interestingly, a starry sky pattern, even in the wall of the cecum. All layers of the cecum were involved. Peritonitis was secondary to the perforation of the colon due to tumor cell infiltration; this further accelerated the demise of the patient.

The salient features for laboratory diagnosis of BL are summarized in Table 6.36.4.

Clinical Manifestation

The annual incidence of BL in the endemic area used to be about 20 to 40 times higher than that in nonendemic areas (62). In Africa, BL is seen mostly in boys, and its initial presentation is frequently a jaw lesion (63). The sporadic type has a bimodal age distribution. The first peak is in children and young adults, with a later peak in elderly individuals (64). The initial presentation in this type is frequently an abdominal tumor (70% to 90%) (62). However, the demographic characteristics in Africa are changing, with a decrease in new cases, an increase in patient age, and an increase in the number of abdominal tumors (56). In the Middle East, North Africa, and South America, BL cases exhibit intermediate features of the disease: Some features are similar to African BL, and other features mimic American BL (56). The Far East (Hong Kong and Japan) shows its own forms of BL, distinguishing it from the BL of other areas.

FIGURE 6.36.18 Cytogenetic karyotype shows $t(8;22)(q24;q11.2)$ from a case of BL. (From Sun T, Atlas of Hematologic Neoplasms. Springer, 2009 with permission).





After the outbreak of acquired immunodeficiency syndrome (AIDS), the incidence of BL increased dramatically in the nonendemic area. In HIV-infected patients, its incidence is about 35% to 40% of all cases of malignant lymphoma (65,66), compared with 1% to 2% in the general population (3). Burkitt leukemia has also been encountered in HIV-infected patients (67). Although BL is seldom seen in other immunodeficiency states (5), BL from HIV-infected patients is designated generally as immunodeficiency-associated BL. This clinical subtype shares clinicopathologic features with those cases without HIV infection (55).

Besides the initial presentation, the endemic and sporadic BLs also differ in the distribution of pathologic lesions. Bone marrow involvement is rarer, but central nervous system involvement is more common in the endemic than in the sporadic subtype (68). A leukemic presentation of Burkitt tumor is specific for the sporadic subtype (69). The sporadic form also frequently involves the testis (Fig. 6.36.14), ovary, pleura, cerebrospinal fluid, peripheral lymph nodes, and pharynx in 10% to 20% of patients (10). Sporadic BL may also be presented in the form of primary effusion lymphoma (70).

Earlier literature showed that BLL (most likely DLBCL/BL) was more frequently seen in adults, with no sexual predilection and less frequent extranodal but more frequent bone marrow involvement than BL. In both BLL and BL, the stage at presentation is usually III or IV, and the median survival is within 1 year (71,72). However, the 5-year survival rate of BL is higher than that of BLL from most studies (72). In BL cases, the HIV-infected patient has a significantly shorter overall survival than the patient without HIV infection (55). With the current improvement in the treatment of BL, the prognosis in pediatric cases is excellent. In the early stages of the disease (stages I and II), the event-free survival rate ranges from 85% to 100%, whereas in the advanced stages (III and IV), the survival rate is 75% to 85% (73). In the current literature, DLBCL/

BL cases consistently show worse prognosis than that of BL and DLBCL cases due to their failure to respond to the therapeutic regimens for either of the two entities (9,15).

BL is a highly aggressive tumor, probably due to its short doubling time of 24 hours. Therefore, prompt diagnosis and immediate treatment are required. The therapeutic strategy is a very intensive chemotherapy applied within a short duration. With this new approach, even patients with advanced stage BL are curable (74).

REFERENCES

1. Burkitt DR. A sarcoma involving the jaws in Africa children. *Br J Surg*. 1958;46:218–223.
2. Berard C, O’Conor GT, Thomas GT, et al. Histopathological definition of Burkitt’s tumor. *Bull World Health Organ*. 1969;40:601–607.
3. The Non-Hodgkin’s Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin’s lymphomas. *Cancer*. 1982;49:2112–2135.
4. Harris NL, Jaffe ES, Stein H, et al. A revised European-American Classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
5. Diebold J, Jaffe ES, Raphael M, Warnke RA. Burkitt lymphoma. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:181–184.
6. Magrath IT, Jaffe ES, Bhatia K. Burkitt’s lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001: 953–986.
7. Brady G, MacArthur GJ, Farrell PJ. Epstein-Barr Virus and Burkitt lymphoma. *J Clin Pathol*. 2007;60:1397–1402.
8. Leoncini L, Aphael M, Stein H, et al. Burkitt lymphoma, In: Swerdlow SH, Campo E, Harris NL, et al., eds., *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:262–264.
9. Kluin PM, Harris NL, Stein H, et al. B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:265–266.
10. Medeiros LJ. Intermediate and high-grade diffuse non-Hodgkin’s lymphomas in the Working Formulation. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. 2nd ed. Philadelphia, PA: W.B. Saunders; 1995: 283–343.
11. Wright DH. What is Burkitt’s lymphoma? *J Pathol*. 1997;182:125–127.
12. Brunning RD, McKenna RW. *Tumor of the Bone Marrow*. Washington, DC: Armed Forces Institute of Pathology; 1994:399–400.
13. Payne CM, Grogan TM, Cromey DW, et al. An ultrastructural morphometric and immunophenotypic evaluation of Burkitt’s and Burkitt’s-like lymphomas. *Lab Invest*. 1987;57:200–218.
14. Davi F, Delecluse HJ, Cuiet P, et al. Burkitt-like lymphoma in AIDS patients. Characterization within a series of 103 human immunodeficiency virus-associated non-Hodgkin’s lymphomas. *J Clin Oncol*. 1998;16:3788–3795.

TABLE 6.36.4
Salient Features for Laboratory Diagnosis of Burkitt Lymphoma
• Monoclonal surface Ig pattern • Positive B-cell antigens: CD19, CD20, CD22, CD79a, PAX5 • Specific markers: Ki-67 (>99% cells), CD10, bcl-6, c-myc protein, CD21 (in endemic type) • Negative markers: CD5, CD23, bcl-2, IRF4/MUM1, TdT • Cytogenetic karyotypes: t(8;14), t(8;22), t(2;8) • Molecular markers: MYC/IgH, MYC/IgK, MYC/IgL translocations • EBV genome: Present in 100% endemic subtype, 11%–20% sporadic subtype, 20%–40% immunodeficiency-associated subtype

EBV, Epstein–Barr virus; CD, cluster of differentiation; TdT, terminal deoxynucleotidyl transferase; Ig, immunoglobulin.

15. Carbone A, Gloghini A, Aiello A, et al. B-cell lymphomas with features intermediate between distinct pathologic entities. From pathogenesis to pathology. *Hum Pathol*. 2010;41:621–631.
16. Frost M, Newell J, Lones MA, et al. Comparative immunohistochemical analysis of pediatric Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Clin Pathol*. 2004;121:384–392.
17. Natkunam Y, Lossos IS, Taidi B, et al. Expression of the human germinal center-associated lymphoma (HGAL) protein, a new marker of germinal center B-cell derivation. *Blood*. 2005;105:3979–3986.
18. Nasr MR, Rosenthal N, Syrbu S. Expression profiling of transcription factors in B- or T-acute lymphoblastic leukemia/lymphoma and Burkitt lymphoma: Usefulness of PAX5 immunostaining as pan-pre-B-cell marker. *Am J Clin Pathol*. 2010;133:41–48.
19. Xu Y, McKenna RW, Molberg KH, et al. Clinicopathologic analysis of CD10+ and CD10– diffuse large B-cell lymphoma: identification of a high-risk subset with coexpression of CD10 and bcl-2. *Am J Clin Pathol*. 2001;116:183–190.
20. Schniederjan SD, Li S, Saxe DF, et al. A novel flow cytometric antibody panel for distinguishing Burkitt lymphoma from CD10+ diffuse large B-cell lymphoma. *Am J Clin Pathol*. 2010;133:718–726.
21. Rodig SJ, Vergilio JA, Shahsafaei A, et al. Characteristic expression patterns of TCL-1, CD38, and CD44 identify aggressive lymphomas harboring a MYC translocation. *Am J Surg Pathol*. 2008;32:113–122.
22. Aiello A, Delia D, Fontanella E, et al. Expression of differentiation and adhesion molecules in sporadic Burkitt's lymphoma. *Hematol Oncol*. 1990;8:229–238.
23. Spina D, Leoncini T, Megha T, et al. Cellular kinetic and phenotypic heterogeneity in and among Burkitt's and Burkitt-like lymphomas. *J Pathol*. 1997;182:145–150.
24. Gloghini A, De Paoli P, Gaidano G, et al. High frequency of CD45RO expression in AIDS-related B-cell non-Hodgkin's lymphomas. *Am J Clin Pathol*. 1995;104:680–688.
25. Lin CW, O'Brien S, Faber J, et al. De novo CD5+ Burkitt's lymphoma/leukemia. *Am J Clin Pathol*. 1999;112:828–835.
26. Magrath I. The pathogenesis of Burkitt's lymphoma. *Adv Cancer Res*. 1990;55:133–270.
27. Taub R, Moulding C, Battey J, et al. Activation and somatic mutation of the translocated c-myc gene in Burkitt's lymphoma cells. *Cell*. 1984;36:339–348.
28. May PC, Foot N, Dunn R, et al. Detection of cryptic and variant IGH-MYC rearrangements in high-grade non-Hodgkin's lymphoma by fluorescence in situ hybridization: implications for cytogenetic testing. *Cancer Genet Cytogenet*. 2010;198:71–75.
29. Akasaka T, Akasaka H, Ueda C, et al. Molecular and clinical features of non-Burkitt's, diffuse large-cell lymphoma of B-cell type associated with the c-myc/immunoglobulin heavy-chain fusion gene. *J Clin Oncol*. 2000;18:510–518.
30. Vaishampayan UN, Mohamed AN, Dugan MC, et al. Blastic mantle cell lymphoma with Burkitt-type translocation and hypodiploidy. *Br J Haematol*. 2002;115:66–68.
31. Shou Y, Martelli ML, Gabrea A, et al. Diverse karyotypic abnormalities of the c-myc locus associated with c-myc dysregulation and tumor progression in multiple myeloma. *Proc Natl Acad Sci U S A*. 2000;97:228–233.
32. Slavutsk I, Andreoli G, Gutierrez M, et al. Variant (8;22) translocation in lymphoblastic lymphoma. *Leuk Lymphoma*. 1996;21:169–172.
33. Arranz E, Martinez B, Richart A, et al. Increased c-myc oncogene copy number detected with combined modified comparative genomic hybridization and FISH analysis in a Richter syndrome case with complex karyotype. *Cancer Genet Cytogenet*. 1988;106:80–83.
34. Merchant S, Schlette E, Sanger W, et al. Mature B-cell leukemias with more than 55% prolymphocytes: report of 2 cases with Burkitt lymphoma-type chromosomal translocations involving c-myc. *Arch Pathol Lab Med*. 2003;127:305–309.
35. Siebert R, Mathiesen P, Harder S, et al. Application of interphase fluorescence in situ hybridization for the detection of the Burkitt translocation t(8;14) (q24;q32) in B-cell lymphomas. *Blood*. 1998;91:984–990.
36. Leucci E, Cocco M, Onnis A, et al. MYC translocation-negative classical Burkitt lymphoma cases: an alternative pathogenetic mechanism involving miRNA deregulation. *J Pathol*. 2008;216:440–450.
37. Robertus JL, Kuiver J, Weggemans C, et al. MiRNA profiling in B non-Hodgkin lymphoma: a MYC-related miRNA profile characterizes Burkitt lymphoma. *Br J Haematol*. 2010;149:896–918.
38. Shiramizu B, Barriga F, Neequaye J, et al. Patterns of chromosomal breakpoint location in Burkitt's lymphoma. Relevance to geography and Epstein-Barr virus association. *Blood*. 1991;77:1516–1526.
39. Pelicci PG, Knowles D, Magrath I, et al. Chromosomal breakpoint and structural alterations of the c-myc locus differ in endemic and sporadic forms of Burkitt lymphoma. *Proc Natl Acad Sci U S A*. 1986;83:2984–2988.
40. Neri A, Barriga F, Knowles DM, et al. Different regions of the immunoglobulin heavy-chain locus are involved in chromosomal translocations in distinct pathogenetic forms of Burkitt lymphoma. *Proc Natl Acad Sci U S A*. 1988;85:2748–2752.
41. Gutierrez MI, Bhatia K, Barriga F, et al. Molecular epidemiology of Burkitt's lymphoma from South America: differences in break-point location and Epstein-Barr virus association from tumors in other world regions. *Blood*. 1992;79:3261–3266.
42. Ladanyi M, Offit K, Jhanwar SC, et al. MYC rearrangement and translocations involving band 8q24 in diffuse large cell lymphomas. *Blood*. 1991;77:1057–1063.
43. Gaidano G, Pastore C, Gloghini A, et al. Genetic heterogeneity of AIDS-related small non-cleaved lymphoma. *Br J Haematol*. 1997;98:726–732.
44. Yano T, Van Krieken JH, Magrath IT, et al. Histogenetic correlations between subcategories of small noncleaved cell lymphomas. *Blood*. 1992;79:1282–1290.
45. Velangi MR, Reid MM, Bown N, et al. Acute lymphoblastic leukaemia of the L3 subtype in adults in the Northern health region of England 1983–99. *J Clin Pathol*. 2002;55:591–595.
46. Snuderl M, Kolman OK, Chen YB, et al. B-cell lymphomas with concurrent IGH-BCL-2 and MYC rearrangements are aggressive neoplasms with clinical and pathologic features distinct from Burkitt lymphoma and diffuse large B-cell lymphoma. *Am J Surg Pathol*. 2010;34:327–340.
47. Seegmiller AC, Garcia R, Huang R, et al. Simple karyotype and bcl-6 expression predict a diagnosis of Burkitt lymphoma and better survival in IG-MYC rearranged high-grade B-cell lymphoma. *Mod Pathol*. 2010;23(7):909–920.
48. Carbone A, Gloghini A, Gaidano G, et al. AIDS-related Burkitt's lymphoma. Morphologic and immunophenotypic study of biopsy specimens. *Am J Clin Pathol*. 1995;103:561–567.
49. Preudhomme C, Dervite I, Wattel E, et al. Clinical significance of p53 mutations in newly diagnosed Burkitt's lymphoma and acute lymphoblastic leukemia: a report of 48 cases. *J Clin Oncol*. 1995;13:812–820.
50. Cherney BW, Bhatia K, Sgadari C, et al. Role of the p53 suppressor gene in the tumorigenicity of Burkitt's lymphoma cells. *Cancer Res*. 1997;57:2508–2515.

51. Bellan C, Lazzi S, De Falco G, et al. Burkitt's lymphoma: new insights into molecular pathogenesis. *J Clin Pathol*. 2003;56:188–193.
52. Hummel M, Bentink S, Berger H, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. *N Engl J Med*. 2006;354:2419–2430.
53. Dave SS, Fu K, Wright GW, et al. Molecular diagnosis of Burkitt lymphoma. *N Engl J Med*. 2006;354:2431–2442.
54. Epstein MA. Historical backgrounds: Burkitt's lymphoma and Epstein-Barr virus. In: Lenoir G, O'Connor G, Olweny CL, eds. *Burkitt's Lymphoma: A Human Cancer Model*. Lyon, France: IARC Scientific Publications; 1985:17–27.
55. Spina M, Tirelli U, Zagonel V, et al. Burkitt's lymphoma in adults with and without human immunodeficiency virus infection. *Cancer*. 1998;82:766–774.
56. Shapira J, Peylan-Ramu N. Burkitt's lymphoma. *Oral Oncol*. 1998;34:15–23.
57. Tao G, Robertson KD, Manns A, et al. Epstein-Barr virus (EBV) in endemic Burkitt's lymphoma. Molecular analysis of primary tumor tissue. *Blood*. 1998;91:1373–1381.
58. Chao TY, Wang TY, Lee WH. Association between Epstein-Barr virus and Burkitt's lymphoma in Taiwan. *Cancer*. 1997;80:121–128.
59. Neri A, Barriga F, Inghirami G, et al. Epstein-Barr virus infection precedes clonal expansion in Burkitt's and acquired immunodeficiency syndrome-associated lymphoma. *Blood*. 1991;77:1092–1095.
60. Hamilton-Dutoit SJ, Pallesen G. A survey of Epstein-Barr virus gene expression in sporadic non-Hodgkin's lymphomas. Detection of Epstein-Barr virus in a subset of peripheral T-cell lymphomas. *Am J Pathol*. 1992;140:1315–1325.
61. Kieff E, Leibowitz D. Oncogenesis by herpesvirus. In: Weinberg RA, ed. *Oncogenes and the Molecular Origin of Cancer*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1989:259.
62. Philip T. Burkitt's lymphoma in Europe. In: Lenoir GM, O'Connor GT, Olweny CLM, eds. *Burkitt's Lymphoma: A Human Cancer Model*. London: IARC Scientific Publications; 1985:107–118.
63. Aboulola M, Boukheloua B, Labjadj Y, et al. Burkitt's lymphoma in Algeria. In: Lenoir GM, O'Connor GT, Olweny CLM, eds. *Burkitt's Lymphoma: A Human Cancer Model*. London: IARC Scientific Publications; 1985:99–105.
64. Pavlova Z, Parker JW, Taylor CR, et al. Small noncleaved follicular center cell lymphoma: Burkitt's and non-Burkitt's variants in the US. 2. Pathologic and immunologic features. *Cancer*. 1987;59:1892–1902.
65. Knowles DM, Chamulak GA, Subar M, et al. Clinicopathologic, immunophenotypic, and molecular genetic analysis of AIDS-associated lymphoid neoplasia. Clinical and biologic implications. *Pathol Ann*. 1988;23(Pt 2):33–67.
66. Levine AM. AIDS-associated malignant lymphoma. *Med Clin North Am*. 1992;76:253–267.
67. Fenaux P, Lai JL, Miaux O, et al. Burkitt cell acute leukemia (L3 ALL) in adults. A report of 18 cases. *Br J Haematol*. 1989;71:371–376.
68. Bouffet E, Frappaz D, Pinkerton R, et al. Burkitt's lymphoma. A model for clinical oncology. *Eur J Cancer*. 1991;27:504–509.
69. Lemerle J. Rapporteur's report. In: Lenoir GM, O'Connor GT, Olweny CLM, eds. *Burkitt's Lymphoma: A Human Cancer Model*. Lyon, France: IARC Scientific Publications; 1985:149–151.
70. Asoli V, Coco FL, Attini M, et al. Primary effusion Burkitt's lymphoma with t(8;22) in a patient with hepatitis C virus-related cirrhosis. *Hum Pathol*. 1997;28:101–104.
71. Levine AM, Pavlova Z, Pockros AW, et al. Small noncleaved follicular center cell (FCC) lymphoma. Burkitt and non-Burkitt variant in the United States. 1. Clinical features. *Cancer*. 1983;52:1073–1079.
72. Miliauskas JR, Berard CW, Young RC, et al. Undifferentiated non-Hodgkin's lymphomas (Burkitt's and non-Burkitt's type). The relevance of making this histologic distinction. *Cancer*. 1982;50:2115–2121.
73. Sandlund JT, Downing JR, Crist WM. Non-Hodgkin's lymphoma in children. *N Engl J Med*. 1996;334:1238–1248.
74. Divine M, Casassus P, Koscielny S, et al. Small non-cleaved cell lymphoma. A prospective multicenter study of 51 adults treated with the LMB pediatric protocol. *Blood*. 1999;10(suppl 1):523a.

CASE 37

T-Cell Large Granular Lymphocytic Leukemia

CASE HISTORY

A 77-year-old man with a history of cyclic neutropenia was admitted to the hospital because of worsening neutropenia. The patient was found to have pancytopenia 9 years prior to the current admission during an investigation of his cardiac problem. At that time, his total leukocyte count was 3,900/mL with 73% lymphocytes and 11% neutrophils. The hematocrit was 38.7%, hemoglobin 13.3 g/dL, and platelets 93,000/mL. The patient had not been treated despite cyclic drops of all cell counts. He had no major infections except for diverticulitis. His arthritis was considered nonspecific.

On admission, no lymphadenopathy or hepatosplenomegaly was found. An automated complete blood count revealed a total leukocyte count of 1,900/mL with 5.4% neutrophils and 72% lymphocytes. His hematocrit was 38%, hemoglobin 13 g/dL, and platelets 130,000/mL. Moderate numbers of large granular lymphocytes (LGLs) were found on the peripheral blood smear.

A bone marrow biopsy was performed and showed a marked decrease of myeloid cells with a myeloid to erythroid precursor (M/E) ratio of 0.7:1. The differential count revealed marked maturation arrest at the myelocytic stage (14.5% myelocytes, 0.5% metamyelocytes, 1% bands, and

2% segmented neutrophils) and an increase in lymphocytes (36%). A small number of LGLs were also identified. Multiple lymphoid aggregates as well as interstitial lymphoid infiltration were found in the core biopsy.

The patient was treated with methotrexate and prednisone. At the time of discharge, the total leukocyte count was 2,400/mL with 43% neutrophils, 30% lymphocytes, and 25% monocytes. The hematocrit was 39.2%, hemoglobin 13.5 g/dL, and platelets 247,000/mL. The patient was treated with the same regimen continuously on an outpatient basis.

FLOW CYTOMETRY FINDINGS

The bone marrow biopsy revealed 98% CD2, 97% CD3, 96% CD5, 23% CD3/CD4, 81% CD3/CD8, 3% CD19, 6% CD20, 5% k, 4% l, 35% CD16, 51% CD3/CD57, and 2% CD3/CD56 (Fig. 6.37.1).

MOLECULAR GENETIC FINDINGS

T-cell receptor gene rearrangement analysis of the bone marrow showed T-cell receptor b-chain gene rearrangement. Cytogenetic study revealed a normal male karyotype of 46, XY.

DISCUSSION

Large granular lymphoproliferative disorder (LGLD) was first reported by Brouet et al. (1) in 1975 and was defined by McKenna et al. (2) in 1977 to be a distinct clinicopathologic entity. However, LGLD as defined in the early literature was composed of a highly heterogeneous group, which includes chronic leukemia, acute leukemia, lymphoma, and reactive lymphocytosis. In terms of cell lineage, it contains tumor cells of the natural killer (NK) cell lineage or the NK-like T-cell lineage. It is, therefore, not surprising to find the wide clinical spectrum, from indolent to highly aggressive, in the early reported cases. The current definition of LGLD is confined to the leukemic type without lymph node involvement. By this definition, LGLD can be divided into chronic T-large granular lymphocyte (T-LGL) leukemia, chronic lymphoproliferative disorder of NK cells (CLPD-NK), and NK-LGL leukemia, which includes mainly the aggressive NK-cell leukemia (3–5). Chronic LGLD is much more common than the aggressive type. Among the chronic types, 85% of cases are chronic T-LGL leukemia, and 5% of cases are CLPD-NK (5). These two types of chronic LGLD are mainly distinguished by immunophenotypes, but are similar in morphology and clinical manifestations (6,7). The immunophenotypes of these three groups of LGLD are summarized in Table 6.37.1.

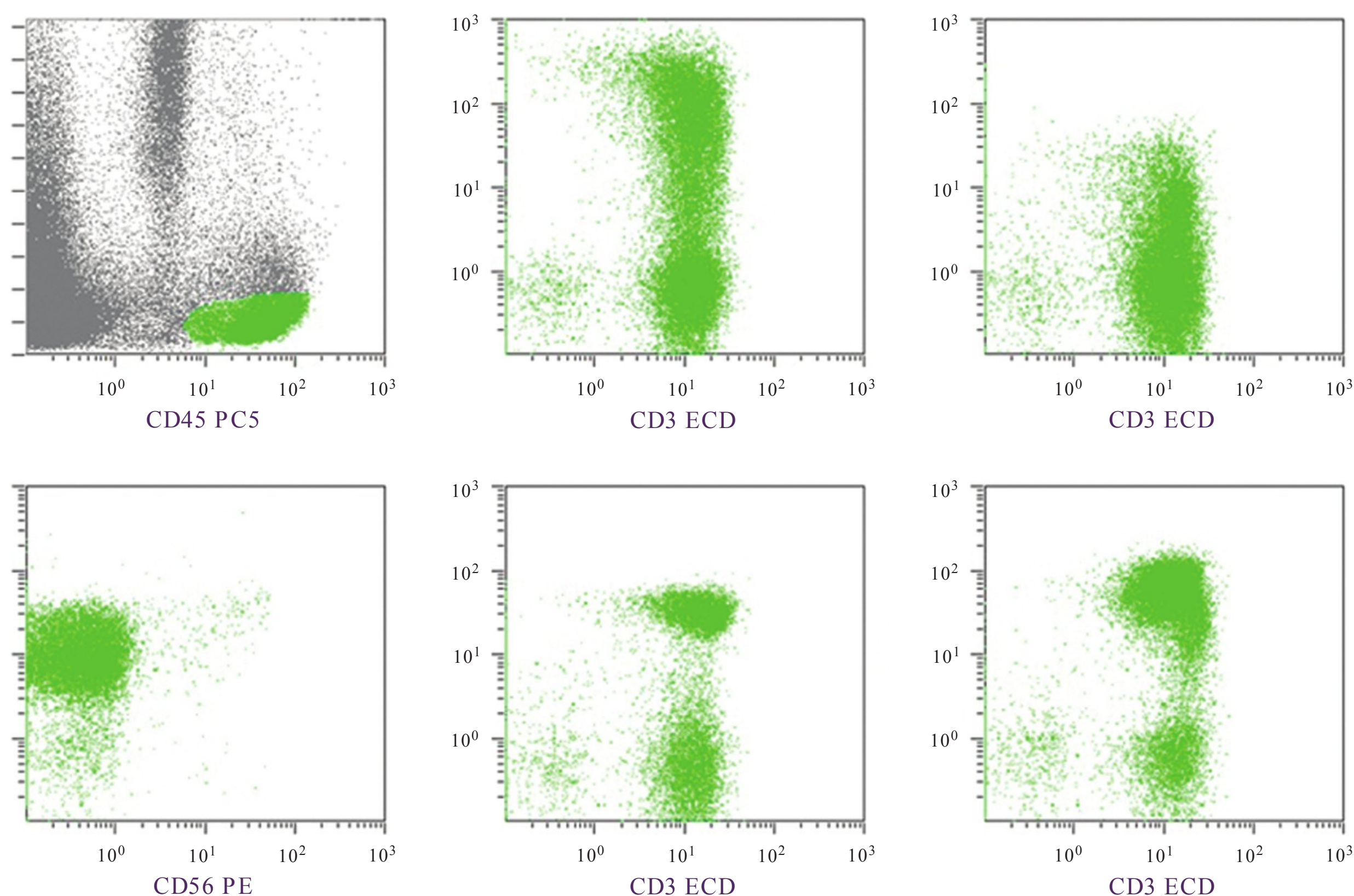


FIGURE 6.37.1 Flow cytometric histograms show positive CD2, CD3, CD57, and CD16 reactions but negative CD56 reaction. There is a reverse CD4/CD8 ratio. The CD4-positive population represents the normal T lymphocytes. SS, side scatter; PC5, phycoerythrin-cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ECD, phycoerythrin-Texas Red.

TABLE 6.37.1			
Major Immunophenotypes of LGLD			
Marker	T-LGL leukemia	CLPD-NK	NK-LGL leukemia
CD3	Positive	Negative	Negative
CD16	Positive	Positive/negative	Positive
CD56	Negative	Weakly positive	Positive
CD57	Positive	Negative	Negative
CD4	Negative	Negative	Negative
CD8	Positive	Negative	Positive/negative
Cytotoxic granule protein	Positive	Positive	Positive
TCR protein	Positive	Negative	Negative
KIR	Positive	Positive	Unknown

CLPD-NK, chronic lymphoproliferative disorder of NK cells; KIR, killer immunoglobulin-like receptor; LGLD, large granular lymphoproliferative disorder; NK, natural killer; TCR, T-cell receptor.

In the World Health Organization (WHO) classification, chronic T-LGLD was renamed T-LGL leukemia, and cases with NK-cell phenotypes were classified as NK disorders (6,7). The WHO classification defines T-LGL leukemia as “a heterogeneous disorder characterized by a persistent (>6 months) increase in the number of peripheral blood LGLs, usually between 2 and 20 × 10⁹/L, without a clearly identified cause.”

Morphology

The morphology of the tumor cells in T-LGL leukemia is generally similar to that of the normal LGLs, but considerable variation in size and morphology of the tumor cells has been reported (8,9). These cells are larger than the small normal lymphocytes, and their size varies from 15 to 18 mm (Figs. 6.37.2 and 6.37.3). The cytoplasm is usually

pale or transparent, containing three or more azurophilic granules, but some LGL may not contain any visible granules. The normal range of LGLs is 200 to 400/mL (or 10% to 15%) among the mononuclear cells in the peripheral blood (8,9). When a patient has >2,000/mL of LGL or LGL is >40% of the lymphocyte fraction for >6 months, T-LGL leukemia should be suspected (10–12). However, some patients may have LGL of <1,000/mL in the peripheral blood (11), and 600/mL has been suggested as the minimal LGL count for diagnosing T-LGL leukemia (13). There is no agreement on the lymphocyte count in T-LGL leukemia, but 5,000/mL is suggested as the cutoff from reactive lymphocytosis (6).

All possible causes of reactive large granular lymphocytosis should be excluded before further studies by phenotyping or genotyping are conducted. The presence of

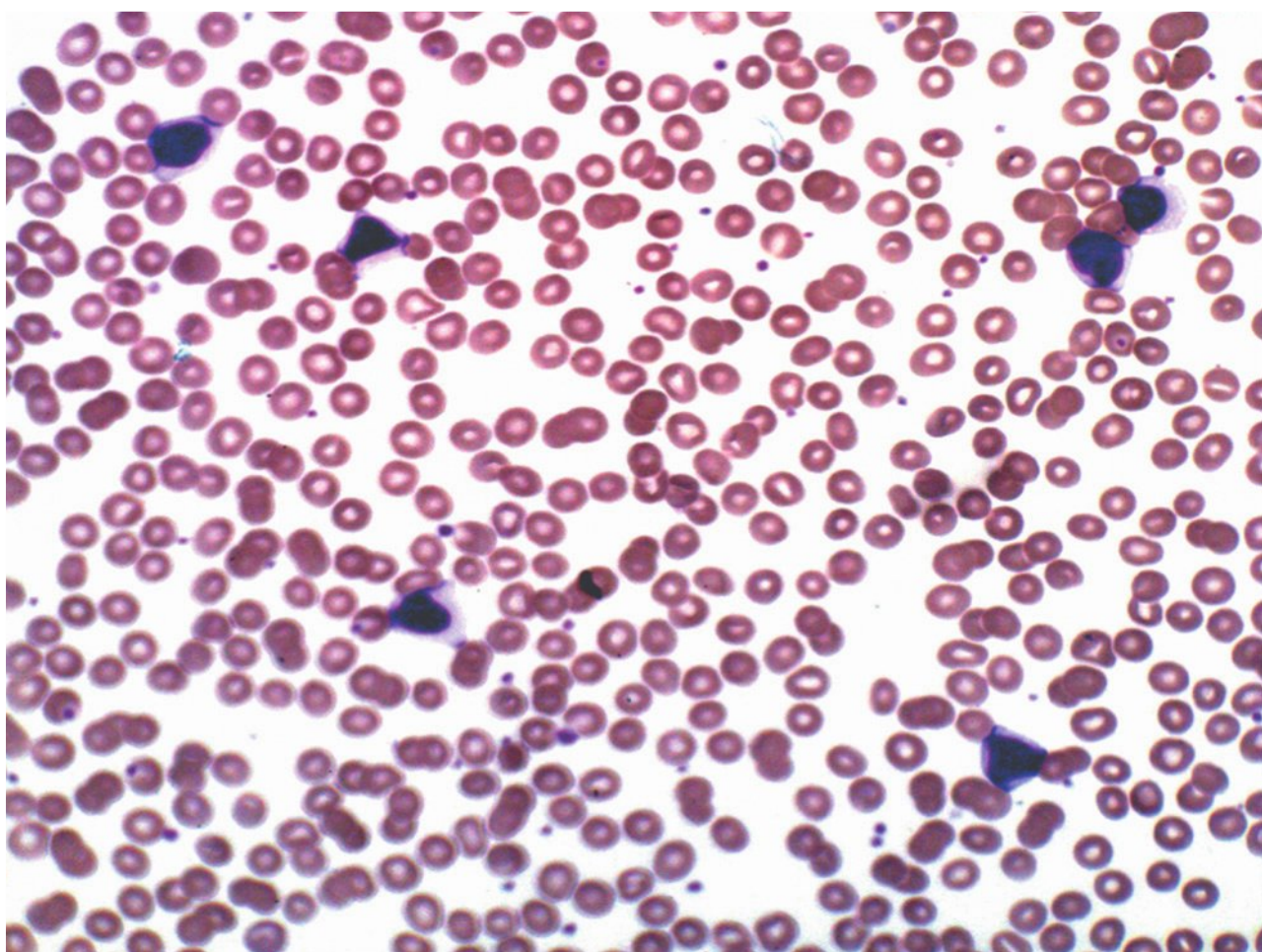


FIGURE 6.37.2 Peripheral blood smear shows several LGLs with abundant transparent cytoplasm. Wright–Giemsa, 40× magnification.

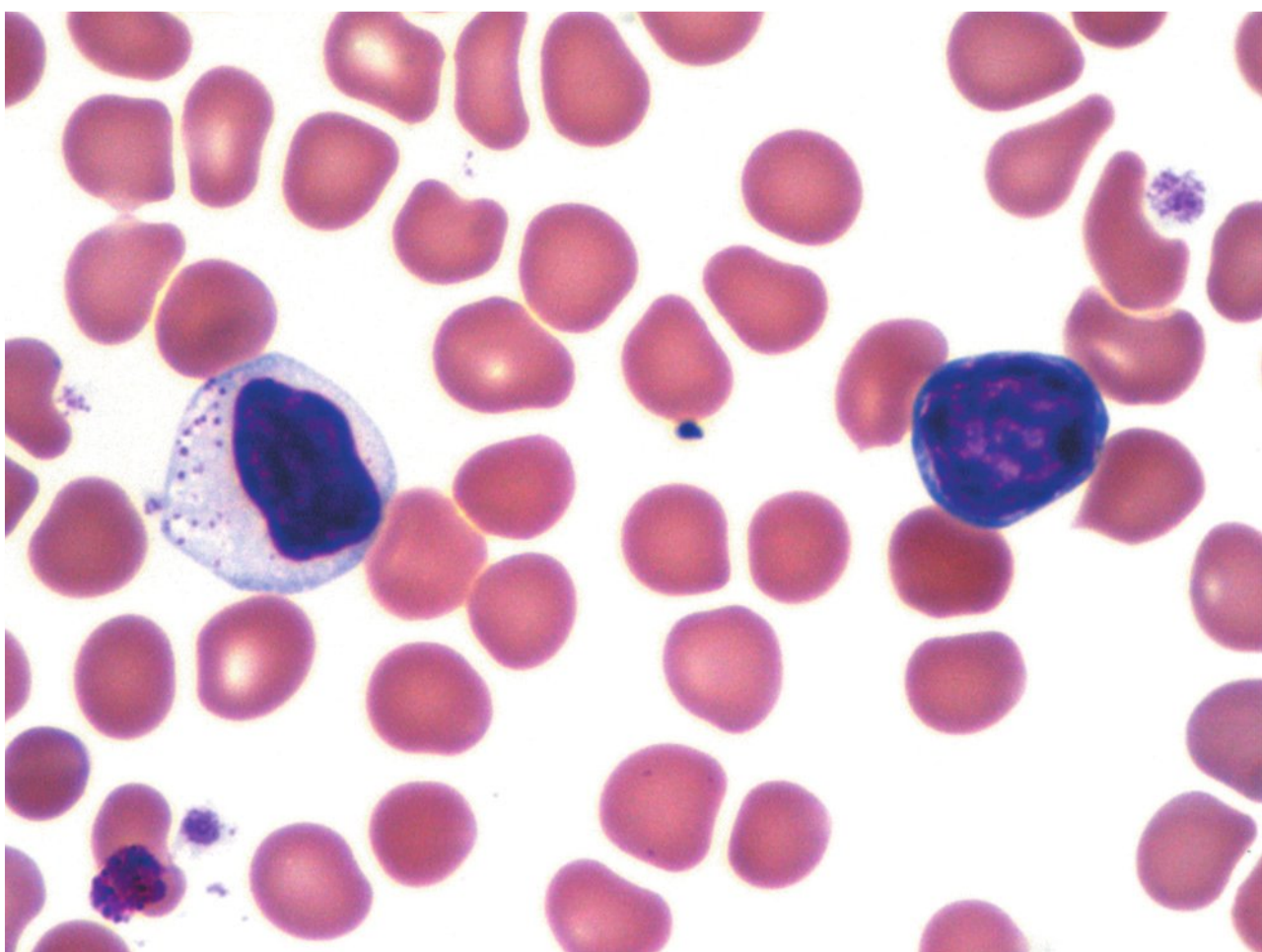


FIGURE 6.37.3 Peripheral blood smear shows one LGL with azurophilic granules in its transparent cytoplasm. A normal lymphocyte is by its side. Wright–Giemsa, 200× magnification.

neutropenia is important for the diagnosis of T-LGL leukemia, because reactive large granular lymphocytosis may have an immunophenotype similar to that of LGL leukemia (14). Anemia and thrombocytopenia are also frequently seen. Anemia in some patients is associated with pure red cell aplasia, which is relatively common in Chinese and Japanese patients with T-LGL leukemia (8). The criteria for pure red cell aplasia are anemia, reticulocytopenia ($<0.1\%$), and selective erythroid hypoplasia and/or aplasia with normal myeloid and megakaryocytic cell lines in the bone marrow.

The bone marrow is frequently involved showing interstitial or nodular lymphocytic infiltration (Fig. 6.37.4). The lymphoid nodule formation is perceived by some authors as a non-neoplastic reaction to the lymphokines secreted by the tumor cells (15). Morice et al. described a linear accumulation of cytotoxic T cells within marrow microvascular structures, which was considered highly specific for T-LGL leukemia (16). The same study showed that the interstitial infiltrates were composed of T-LGL cells, whereas the large lymphoid aggregates were composed of non-neoplastic lymphocytes, a mixed T- and B-cell population. However, immunohistochemical stains are often needed for the identification of the LGLs, particularly because lymphocytic infiltration may be subtle. Some investigators found maturation arrest of the granulocytes and considered this to be the mechanism of neutropenia secondary to lymphokine secretion by the tumor cells (15,17). Bone marrow aspirates may demonstrate LGLs (Fig. 6.37.5), but LGLs in the marrow are less voluminous with inconspicuous azurophilic granules (14). It is the immunophenotype that determines the diagnosis (16,18).

The spleen usually shows leukemic infiltration of the red pulp and prominent reactive germinal follicles consisting of polyclonal B cells (4). Unlike other red pulp involving

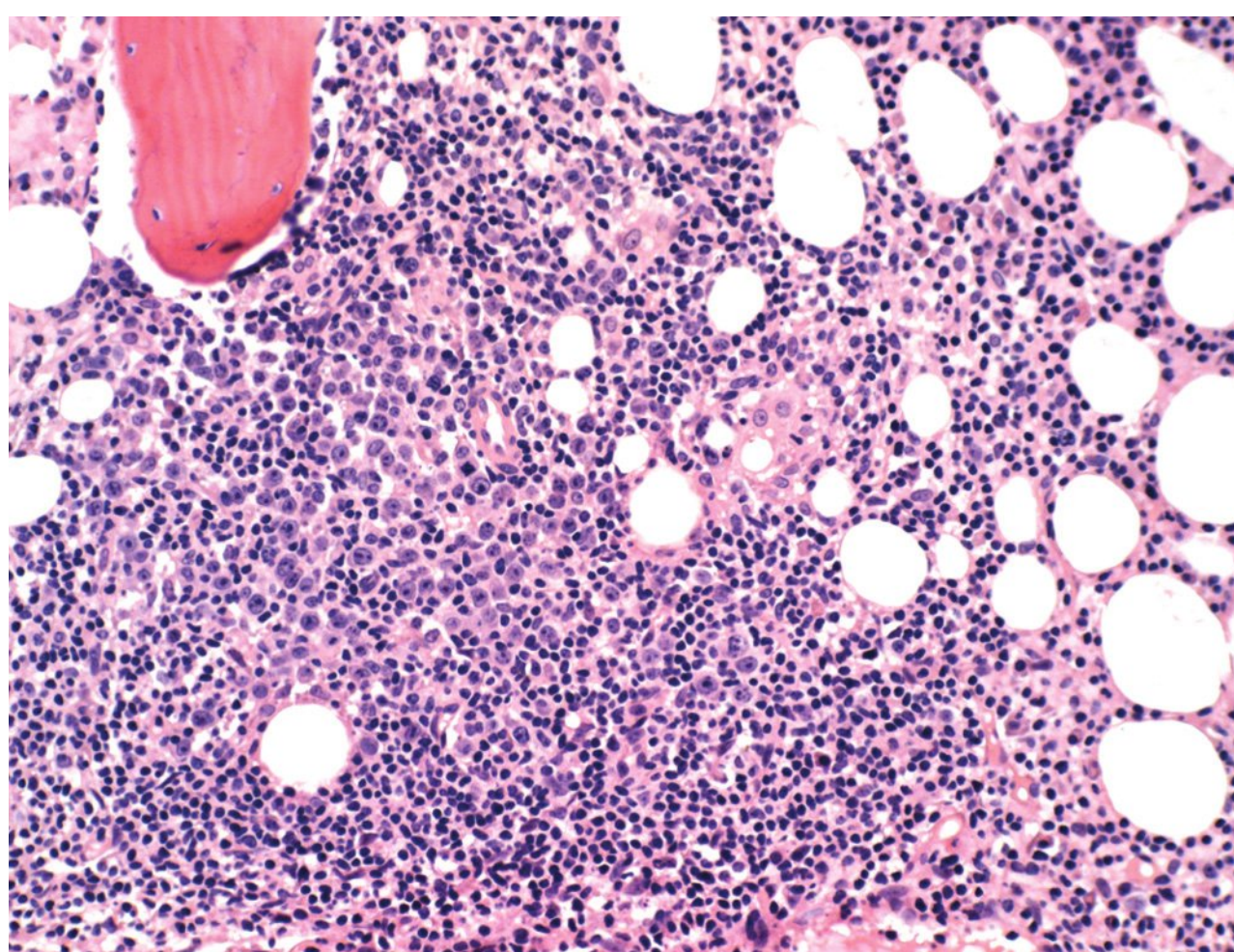


FIGURE 6.37.4 Bone marrow core biopsy reveals a lymphoid aggregate with a pale germinal center and interstitial lymphoid infiltration in the adjacent area. Immunohistochemical stains identify the lymphoid aggregate as reactive and the interstitial infiltrate as T-LGLs. Hematoxylin and eosin, 20× magnification.

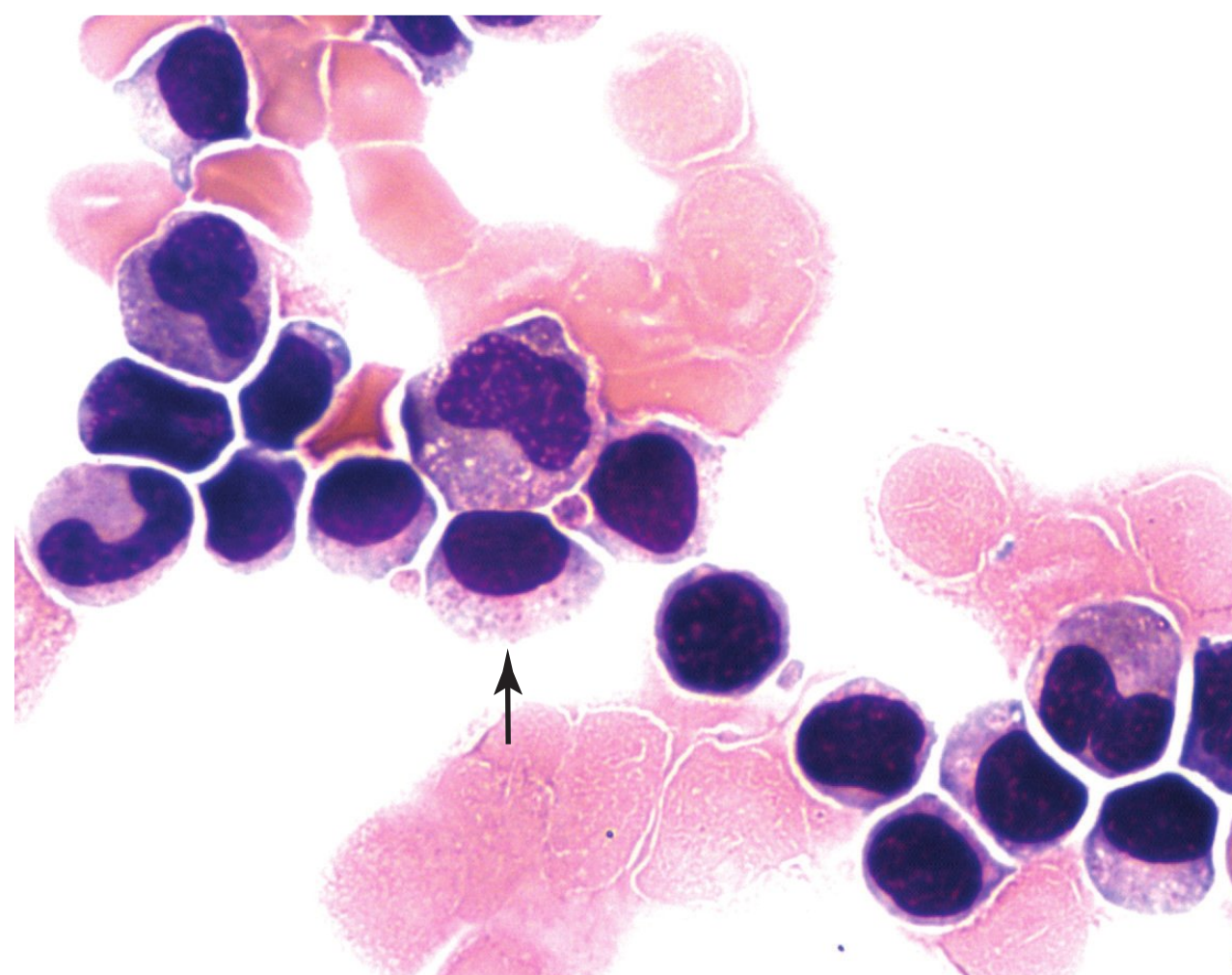


FIGURE 6.37.5 Bone marrow aspirate shows a few LGLs (arrow) that have less voluminous cytoplasm and inconspicuous cytoplasmic granules. Wright-Giemsa, 100× magnification.

leukemias, such as T-prolymphocytic leukemia and chronic lymphocytic leukemia, cells from T-LGL leukemia do not invade the white pulp, so the follicles may show germinal center hyperplasia and expansion of the mantle zones (15). In the liver, lymphoid infiltration is mainly seen in hepatic sinusoids, but infiltration of the portal areas may also be seen in severe cases (4).

Immunophenotype

The typical immunophenotype is CD3+, CD16+, CD56-, CD57+, CD4-, CD8+ (3,6,9,14). For T-cell receptor (TCR) proteins, most cases express TCR_{αβ}. However, in cases that have a CD3+, CD4-, CD8- phenotype, TCR_{γδ} is expressed (19,20). Rare cases may have a phenotype of CD3+, CD4+, CD8- or CD3+, CD4+, CD8+ (3).

Among other T-cell markers, CD2 is consistently expressed, whereas the expression of CD5 and CD7 is variable. Recent studies have shown that the CD5 level is consistently lower than those of other T-cell markers, whereas CD7 may also be expressed at a lower level in some cases (14). The myeloid markers, CD11b and CD11c, are also frequently expressed in T-LGL cells (21,22). The differences in the phenotype between NK cells and NK-like T cells (T-LGL) are discussed in Case 38.

The T-LGL cells are cytotoxic T cells as evidenced by the presence of cytotoxic granule proteins (T-cell intracellular antigen 1 [TIA-1], granzyme B, and perforin) in their cytoplasm (22). Besides the cytotoxic granule protein, the Fas molecule (CD95) and the Fas ligand (CD178) may also play a role in mediating cell killing in T-LGL leukemia (23). On the other hand, Fas molecule also mediates apoptosis, which is facilitated by interleukin-2 (5). Because there is an interleukin-2 defect in T-LGL leukemia, the tumor cells from most patients are resistant to Fas-induced apoptosis (5). This can be one of the possible mechanisms for tumorigenesis in T-LGL leukemia.

The recent discovery of the NK receptor (NKR) system has great potential in helping to stratify various NK-cell leukemia and T-LGL leukemia. The NKR system includes two groups: killer cell immunoglobulin-like receptors (KIRs) and the C-type-lectin-like receptors that consist of heterodimers of CD94 and NKG2 molecules (14). A recent report by Lundell et al. (14) identified CD94 and at least one of the KIR antigens in their study of 23 cases of T-LGL leukemia.

Comparison of Flow Cytometry and Immunohistochemistry

The advantage of flow cytometry is its capability in distinguishing NK cell from T-LGL by the presence or absence of surface and cytoplasmic CD3. In tissue sections, a positive CD3 staining can be due to the presence of either surface or cytoplasmic CD3 antigen. However, immunohistochemistry can demonstrate the cytotoxic granule proteins, thus facilitating the identification of the T-LGL cells in tissue (9). In the bone marrow, LGLs are best identified by CD8, TIA-1, and granzyme B staining (16,24). Immunohistochemical stains for CD57 and CD56 were found to be noncontributory in one study of 36 cases of T-LGL leukemia (16), but CD57 was considered helpful in another study of 9 cases (Fig. 6.37.6) (24). In the spleen, the T-LGL cells are negative for CD5 and CD45RO, but positive for cytotoxic granule proteins, whereas the normal T cells in the spleen are positive for CD5 and CD45RO, but negative for cytotoxic granule proteins (15).

Molecular Genetics

TCR gene rearrangement demonstrated by either Southern blotting technique (Fig. 6.37.7) or polymerase chain reaction (PCR) is one of the most important criteria for the diagnosis of T-LGL leukemia. In most cases, the TCR_β gene is rearranged (6). When both CD4 and CD8 are negative, such as in cases with CD3+, CD4-, CD8-, CD16+ phenotype, the TCR_γ-chain gene is rearranged (2,20).

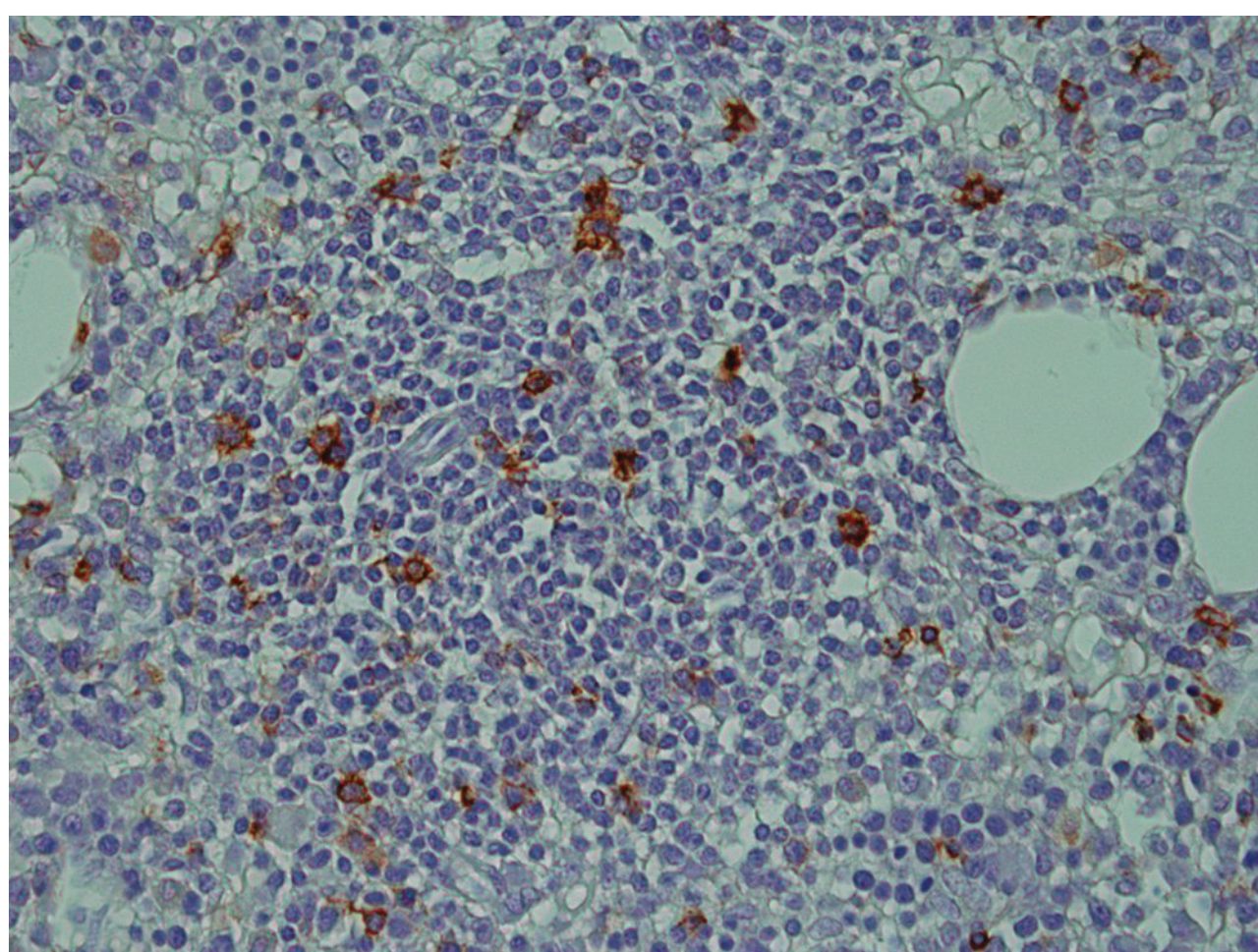


FIGURE 6.37.6 Bone marrow biopsy shows positive CD57 staining in scattered tumor cells. Immunoperoxidase, 40× magnification.

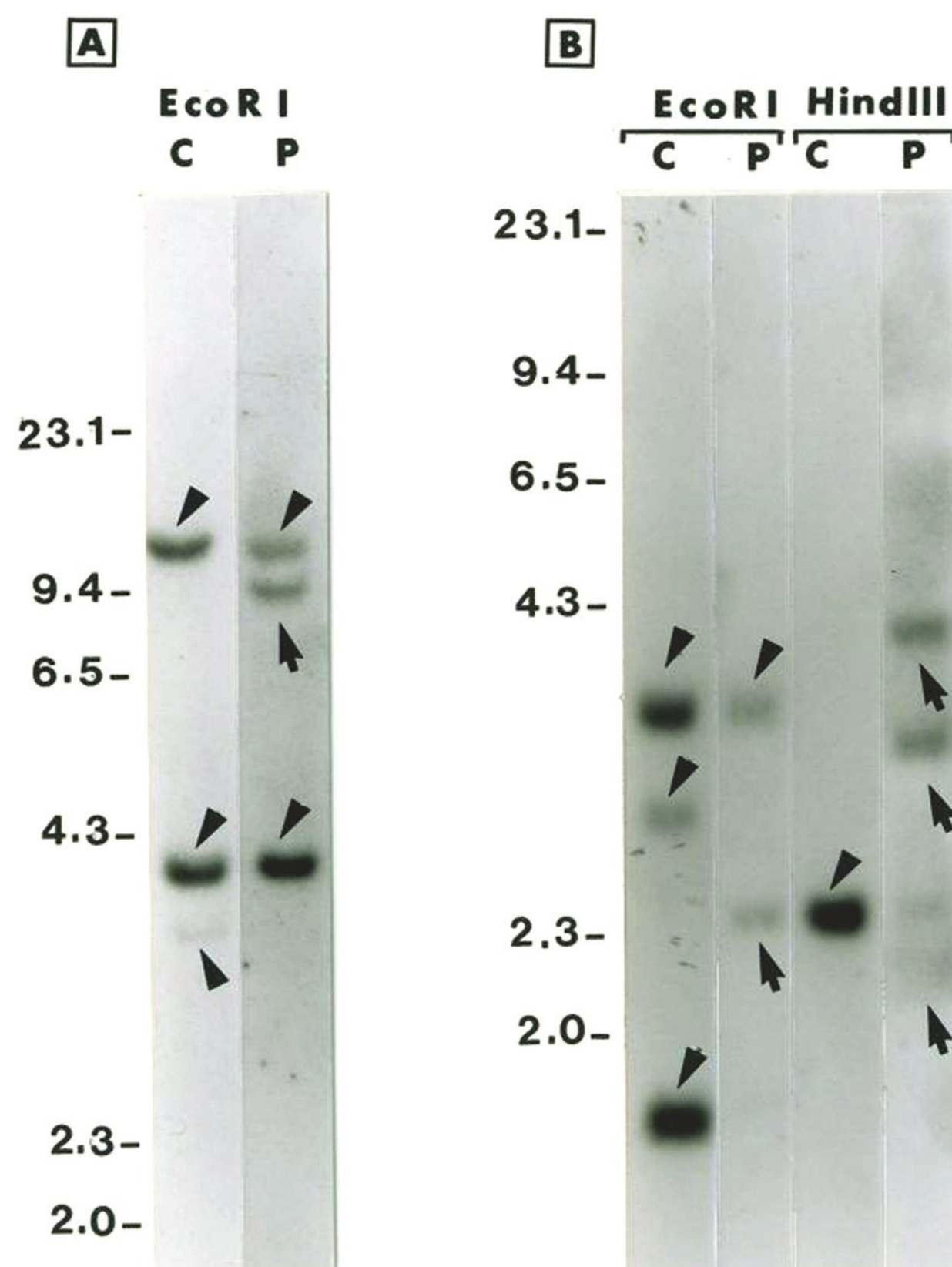


FIGURE 6.37.7 Southern blot hybridization of control (C) and leukemic DNA from the patient (P) with DNA probe for T-cell receptor β (A) and for T-cell receptor γ (B) genes. Rearranged DNA bands in the leukemic DNA are identified by arrows. Germline bands are indicated by arrowheads. (From Sun T, Brody J, Koduru P, et al. Study of the major phenotype of large granular T-cell lymphoproliferative disorder. *Am J Clin Pathol*. 1992;98:516–521, with permission.)

Alternatively, the clonality of T cells can be demonstrated by flow cytometric analysis of the T-cell antigen receptor β -chain variable region (V_{β}) protein expression. One early study of 12 cases of T-LGL leukemia showed a heterogeneous distribution of V_{β} - and J_{β} -chains similar to that seen in normal peripheral blood (5). Another study, in contrast, showed a limited usage of TCR V_{β} -chain in only three families in T-LGL cases (25). A recent study of 23 cases of T-LGL leukemia demonstrated direct or indirect evidence of clonal T-cell populations in 22 cases by analysis of V_{β} expression (14). The most current study confirmed the sensitivity of flow cytometry by comparison with PCR in identification of the T-cell clonality in 19 of 20 cases of T-LGL leukemia, but none of the 18 controls. By contrast, 3/12 controls showed TCR gene rearrangements (26).

Aberrant karyotypes have been reported in only a few cases of T-LGL leukemia. A recent report of two cases is particularly interesting (27). One case showed a novel $inv(7)(p15q22)$ as the sole chromosome abnormality, and the other showed an $inv(14)(q11;q32)$ with evidence of clonal evolution. The breakpoints 7p14–p15 and 14q11 in these cases coincide with the TCR_γ and TCR_α/TCR_δ genes,

respectively. These were the first cases showing TCR gene involvement in aberrant karyotypes of T-LGL cases, and this involvement may represent the mechanism of pathogenesis of T-LGL.

Gene expression profiling study showed profound dysregulation of expression of apoptotic genes and suggested uncoupling of activation and apoptotic pathways as a mechanism for the leukemic cells to escape FAS-mediated apoptosis (28). Pathway-based microarray analysis indicated that balance of proapoptotic and antiapoptotic sphingolipid-mediated signaling was deregulated in T-LGL leukemia cells (28). Emerging evidence suggested that T-LGL leukemia cells with a CD8⁺, CD26[−] phenotype induce such clinical manifestations as neutropenia, anemia, rheumatoid arthritis, and pulmonary artery hypertension through direct destruction of normal tissue (e.g., pulmonary artery endothelial cells and human synovial cells) (29). The enhanced cytotoxic effects in T-LGL leukemia cells are due to elevated levels of activating NKRs as well as their signaling partners, DAP10 and DAP12 (29). The expansion or survival of T-LGL leukemia cells probably also involves the JAK-STAT pathway (30).

The current case is typical for T-LGL leukemia. The patient had an indolent clinical course, being asymptomatic without treatment for 9 years. His major clinical finding was cyclic neutropenia, but recurrent infections did not occur. The immunophenotype of the lymphocytes was CD3⁺, CD8⁺, CD56[−], CD57⁺, but cytotoxic granule proteins were not investigated. TCR_β gene rearrangement established the T-cell clonality of this disease. The patient also had arthritis, but it was not characterized as rheumatoid arthritis. The absence of splenomegaly was atypical, but it does not exclude the diagnosis of T-LGL leukemia. Finally, the patient responded to methotrexate and prednisone therapy. The salient features of laboratory diagnosis of T-LGL leukemia are summarized in Table 6.37.2.

Clinical Manifestations

Large granular lymphocytosis is frequently a reactive process seen in various diseases. The most common causes are viral infections (human immunodeficiency virus and human T-lymphotropic virus type 1) and transplantations (bone marrow and solid organs) (9). It has also been reported in patients with chronic bacterial infections and other neoplastic diseases and in patients postsplenectomy (12,31–33). Therefore, other underlying diseases should be excluded before a leukemic process is considered. In contrast, patients with T-LGL leukemia may be asymptomatic; a clonal disorder should not be ruled out on this basis.

The major clinical manifestations in T-LGL leukemia are chronic large granular lymphocytosis, neutropenia, splenomegaly, and rheumatoid arthritis; therefore, it is difficult to distinguish T-LGL leukemia from Felty syndrome. These two entities are similar in age and sex of the patients and frequency of infections. Furthermore, patients of both diseases have good response to methotrexate. Therefore, some authors consider T-LGL leukemia and Felty syndrome to represent a spectrum of the same disorder (5,9).

TABLE 6.37.2

Salient Features for Laboratory Diagnosis of T-LGL Leukemia

1. Presence of 40% LGLs or an absolute count of >2,000/μL in the peripheral blood for >6 mo without a clearly identified cause*
2. Neutropenia or pancytopenia
3. Rheumatoid factor, antinuclear antibodies, neutrophil antibody, and platelet antibody may or may not be present.
4. Typical immunophenotype: CD3⁺, CD16⁺, CD56[−], CD57⁺, CD4[−], CD8⁺, cytotoxic granule proteins (TIA-1, granzyme B, perforin) ⁺, TCR protein (mostly TCR_{αβ})⁺
5. Genotype: TCR gene but not heavy-chain gene is rearranged.

*The number of LGL cells can be lower than these cut-off points

T-LGL, T-large granular lymphocyte; TCR, T-cell receptor.

Recurrent bacterial infections are secondary to neutropenia. These infections usually involve skin, oropharynx, sinuses, and perirectal areas. Other symptoms may be related to anemia and thrombocytopenia. B symptoms (fever, night sweats, and weight loss) are seen in 20% to 30% of cases (5). Hepatomegaly is seen in 20% of patients. Lymphadenopathy is rare; if present, other subtypes of NK-cell tumors, such as aggressive NK-cell lymphoma/leukemia, should be considered.

Autoimmune antibodies, such as rheumatoid factor, antinuclear antibody, antineutrophil antibody, and antiplatelet antibody, are frequently found in these patients. Interestingly, T-LGL leukemia is occasionally associated with systemic lupus erythematosus, Sjögren syndrome, and Sicca syndrome, in addition to rheumatoid arthritis (5). As mentioned before, T-LGL leukemia patients may also have pure red cell aplasia, which may be due to inhibition of the red cell precursors burst-forming unit and colony-forming unit by LGL clonal expansion (34).

A recent study drew the attention to the differences in clinical spectrum between Asian and Western patients (35). The Western patients have higher incidence of neutropenia, recurrent infection, rheumatoid arthritis, and splenomegaly than Asian patients, while the Asians have higher frequency of pure red cell aplasia. Perhaps because of the low frequencies in neutropenia and recurrent infection, Asian patients also have lower mortality. The authors suggested that antigenic stimuli in these two populations may be different and their treatment should also vary (35). The common treatment in Western countries includes the use of immunosuppressive agents, such as methotrexate, cyclosporine, and steroid with or without growth factors (13). In Asia, the main indication for treatment is pure red cell aplasia, and the use of fludarabine together with mitoxantrone and dexamethasone resulted in high remission rates in Chinese patients (35).

REFERENCES

1. Brouet JC, Sasportes M, Flandrin G, et al. Chronic lymphocytic leukemia of T-cell origin: immunological and clinical evaluation in eleven patients. *Lancet*. 1975;2:890–893.
2. McKenna RW, Parkin J, Kersey JH, et al. Chronic lymphoproliferative disorder with unusual clinical, morphologic, ultrastructural and membrane surface marker characteristics. *Am J Med*. 1997;62:588–596.
3. Loughran TP Jr. Clonal diseases of large granular lymphocytes. *Blood*. 1993;82:1–14.
4. Loughran TP Jr. Large granular lymphocytic leukemia: an overview. *Hosp Pract*. 1998;33:133–138.
5. Lamy T, Loughran TP Jr. Current concepts: large granular lymphocyte leukemia. *Blood Rev*. 1999;13:230–240.
6. Chan WC, Foucar K, Morice WG, et al. T-cell large granular lymphocyte leukaemia. In: Swerdlow SH, Campo E, Harris NL, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:272–273.
7. Villamor N, Morice WG, Chan WC, et al. Chronic lymphoproliferative disorders of NK cells. In: Swerdlow SH, Campo E, Harris NL, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:274–275.
8. Kwong YL, Wong KF. Association of pure red cell aplasia with T large granular lymphocyte leukaemia. *J Clin Pathol*. 1998;51:672–675.
9. Rose MG, Berliner N. T-cell large granular lymphocyte leukemia and related disorders. *Oncologist*. 2004;9:247–258.
10. Tefferi A, Li CY, Witzig TE, et al. Chronic natural killer cell lymphocytosis: a descriptive clinical study. *Blood*. 1994;84:2721–2725.
11. Seminzato G, Zambello R, Starkebaum G, et al. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. *Blood*. 1997;89:256–260.
12. Oshimi K. Lymphoproliferative disorders of natural kill cells. *Int J Hematol*. 1996;63:279–290.
13. Zhou F, Chan WC. T-cell large granular lymphocytic leukemia. In Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:506–512.
14. Lundell R, Hartung L, Hill S, et al. T-cell large granular lymphocyte leukemias have multiple phenotypic abnormalities involving pan-T-cell antigens and receptors for HMC molecules. *Am J Clin Pathol*. 2005;124:937–946.
15. Osuji N, Matutes E, Catovsky D, et al. Histopathology of the spleen in T-cell large granular lymphocyte leukemia and T-cell prolymphocytic leukemia. *Am J Surg Pathol*. 2005;29:935–941.
16. Morice WG, Kurtin PJ, Tefferi A, et al. Distinct bone marrow findings in T-cell large granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. *Blood*. 2002;99:268–274.
17. Agnarsson BA, Loughran TP Jr, Starkebaum G, et al. The pathology of large granular lymphocyte leukemia. *Hum Pathol*. 1989;20:643–651.
18. Sun T, Brody J, Koduru P, et al. Study of the major phenotype of large granular T-cell lymphoproliferative disorder. *Am J Clin Pathol*. 1992;98:516–521.
19. Sun T, Cohen NS, Marino J, et al. CD3+ CD4– CD8– large granular T-cell lymphoproliferative disorder. *Am J Hematol*. 1991;37:173–178.
20. Vie H, Chevalier S, Garand R, et al. Clonal expansion of lymphocytes bearing the gd T-cell receptor in a patient with large granular lymphocyte disorder. *Blood*. 1989;74:285–290.
21. Scott CS, Richards SJ. Classification of large granular lymphocyte (LGL) and NK-associated (NKa) disorders. *Blood Rev*. 1992;6:220–233.
22. Kinney MC. The role of morphologic features, phenotype, genotype, and anatomic site in defining extranodal T-cell or NK-cell neoplasms. *Am J Clin Pathol*. 1999;111(suppl 1):S104–S118.
23. Ng CS, Lo STH, Chan JKC. Peripheral T and putative natural killer cell lymphomas commonly coexpress CD95 and CD95 ligand. *Hum Pathol*. 1999;30:48–53.
24. Evans HL, Burks E, Viswanatha D, et al. Utility of immunohistochemistry in bone marrow evaluation of T-lineage large granular lymphocyte leukemia. *Hum Pathol*. 2000;31:1266–1273.
25. Zambello R, Trentin L, Facco M, et al. Analysis of the T cell receptor in the lymphoproliferative disease of granular lymphocytes: superantigen activation of clonal CD3+ granular lymphocytes. *Cancer Res*. 1995;55:6140–6145.
26. Feng B, Jorgensen JL, Hu Y, et al. TCR-Vb flow cytometric analysis of peripheral blood for assessing clonality and disease burden in patients with T cell large granular lymphocyte leukemia. *J Clin Pathol*. 2010;63:11–146.
27. Wong KF, Chan JCW, Liu HSY, et al. Chromosomal abnormalities in T-cell large granular lymphocyte leukaemia: report of two cases and review of the literature. *Br J Haematol*. 2002;116:598–600.
28. Shah MV, Zhang R, Irby R, et al. Molecular profiling of LGL leukemia reveals role of sphingolipid signaling in survival of cytotoxic lymphocytes. *Blood*. 2008;112:770–781.
29. Chen X, Bai F, Soko L, et al. A critical role for DAP10 and DAP12 in CD8+ T-cell-mediated tissue damage in large granular lymphocyte leukemia. *Blood*. 2009;113:3226–3234.
30. Kirchhoff S, Muller WW, Krueger A, et al. TCR-mediated up-regulation of c-FLIPshort correlates with resistance toward CD95-mediated apoptosis by blocking death-inducing signaling complex activity. *J Immunol*. 2000;165:6293–6300.
31. Ghali V, Castella A, Louis-Charles A, et al. Expansion of large granular lymphocytes (natural killer cells) with limited antigen expression (CD2+, CD3–, CD4–, CD8–, CD16+, NKH-1–) in a human immunodeficiency virus positive homosexual man. *Cancer*. 1990;65:2243–2247.
32. Levitt LJ, Reyes GR, Moonka DK, et al. Human T-cell leukemia virus-I associated T-suppressor cell inhibition of erythropoiesis in a patient with pure red cell aplasia and chronic T-lymphoproliferative disease. *J Clin Invest*. 1988;81:538–548.
33. Scott CS, Richard SH, Sivakumaran M, et al. Transient and persistent expression of large granular lymphocytes (LGL) and NK-associated (NKa) cells: the Yorkshire Leukaemia Group study. *Br J Haematol*. 1993;83:504–515.
34. Hara T, Mizuno Y, Nagata M, et al. Human gd T-cell receptor-positive cell-mediated inhibition of erythropoiesis in vitro in a patient with type 1 autoimmune polyglandular syndrome and pure red cell aplasia. *Blood*. 1990;75:941–950.
35. Kwong YL, Au WY, Leung AYH, et al. T-cell large granular lymphocyte leukemia: an Asian perspective. *Ann Hematol*. 2010;89:331–339.

CASE 38

Natural Killer Cell Lymphoma/Leukemia

CASE HISTORY

A 62-year-old man presented with a history of gunshot wound to his head, which occurred a few years ago, with residual left upper extremity weakness, seizure disorder, and right eye blindness. He was admitted to the hospital because of purulent nasal drainage and increasing periorbital swelling and edema for 2 weeks. Physical examination on admission showed right periorbital swelling with greenish nasal discharge from the left nostril and blood in the right nostril. Computed tomography scan of the maxillary sinuses revealed extensive swelling and enhancement in the soft tissue of the periorbital region with fluid accumulation in both sides of the maxillary sinuses.

The patient was treated with antibiotics and became afebrile. In addition, his facial cellulitis diminished. However, he developed a necrotic area on his skin overlying the right maxillary sinus, and the area was severely infected. Because of the failure of his infection to become completely clear, a biopsy of the necrotic tissue was taken.

Biopsy of the facial tissue showed extensive necrosis, hemorrhages, prominent blood vessels, and multifocal cellular infiltration (Fig. 6.38.1). Higher magnification revealed thrombosis in some blood vessels, fibrinoid necrosis on the vessel wall (Fig. 6.38.2), and perivascular coagulative necrosis in other vessels. Well-confined perivascular infiltrations, the so-called angiocentric pattern, were prominent. Angioinvasive and angiodestructive lesions were also present. The major cellular components in the

infiltrates were atypical lymphocytes with a small number of eosinophils. Neutrophils and plasma cells were not demonstrated. Most of the lymphoid cells had an angulated, convoluted, or cleaved nuclear contour.

FLOW CYTOMETRY

Flow cytometric analysis of the biopsy showed essentially negative B-cell markers: CD19 1%, CD23 0%, FMC-7 0%, k 3%, l 2%. T-cell marker analysis revealed CD3 7%, CD3/CD4 4%, CD3/CD8 3%, CD5 6%, CD7 25%, CD8 26%, CD25 0%. Natural killer (NK)-cell marker study demonstrated CD16-CD56 93%, CD57 11%. Monocyte markers were CD14 1% and CD11c 86% (Fig. 6.38.3).

IMMUNOHISTOCHEMISTRY

Immunohistochemical stains of the biopsy showed negative cytokeratin but positive CD45 (leukocyte common antigen) staining for the lymphoid cells. Further studies with lymphoid markers revealed negative CD20 (B-cell marker) and positive CD3 (T-cell marker) (Fig. 6.38.4). In the T-cell subset study, CD8 was demonstrated exclusively in the lymphoid cells, and CD4 was entirely absent. Based on these findings, NK-cell markers were analyzed; analysis showed a positive staining of CD56 (Fig. 6.38.5) but negative staining of CD57 (Fig. 6.38.6) on the lymphoid cells.

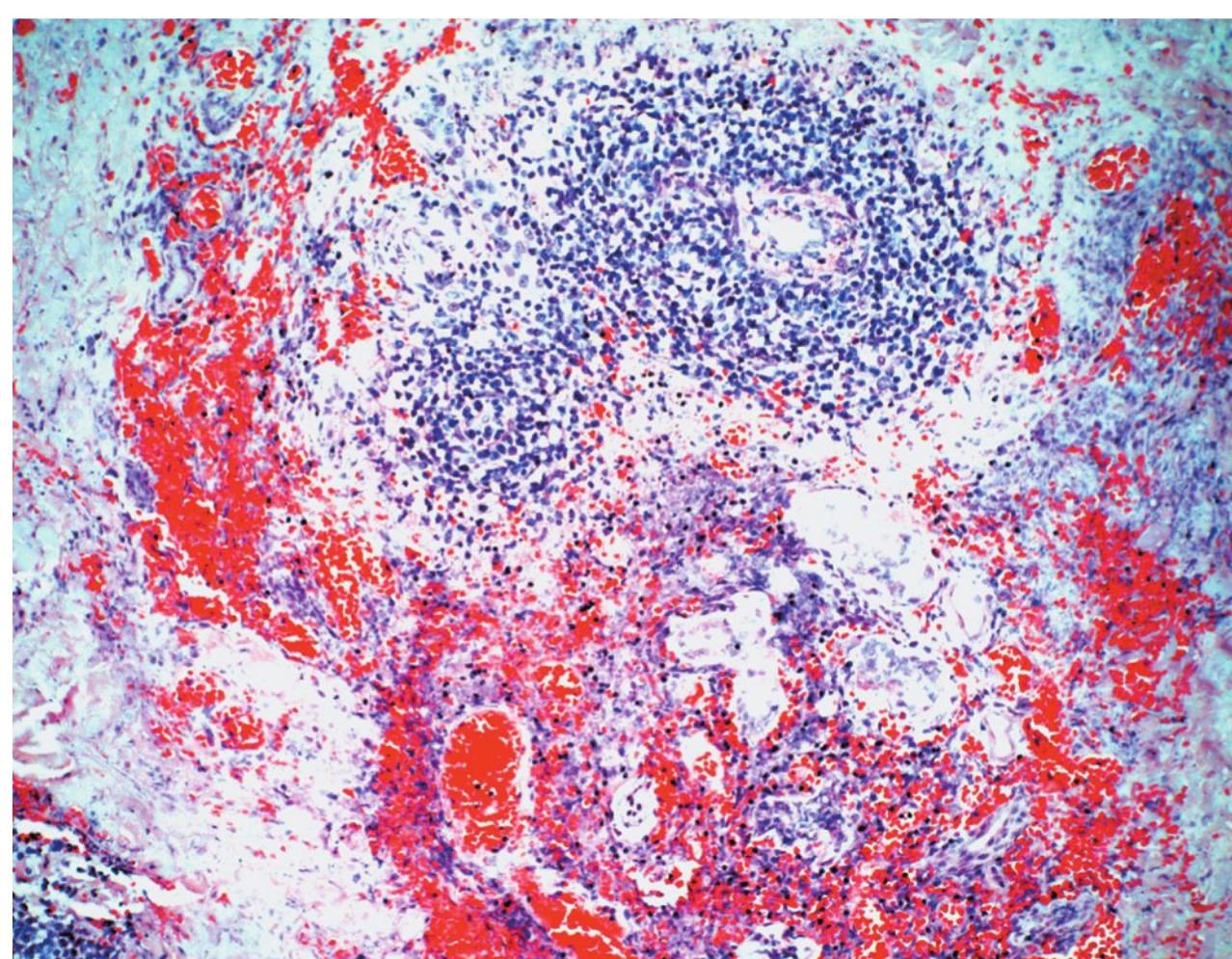


FIGURE 6.38.1 Case of extranodal NK- and/or T-cell lymphoma shows angiocentric lesion with zonal necrosis and hemorrhages. Hematoxylin and eosin, 20× magnification.

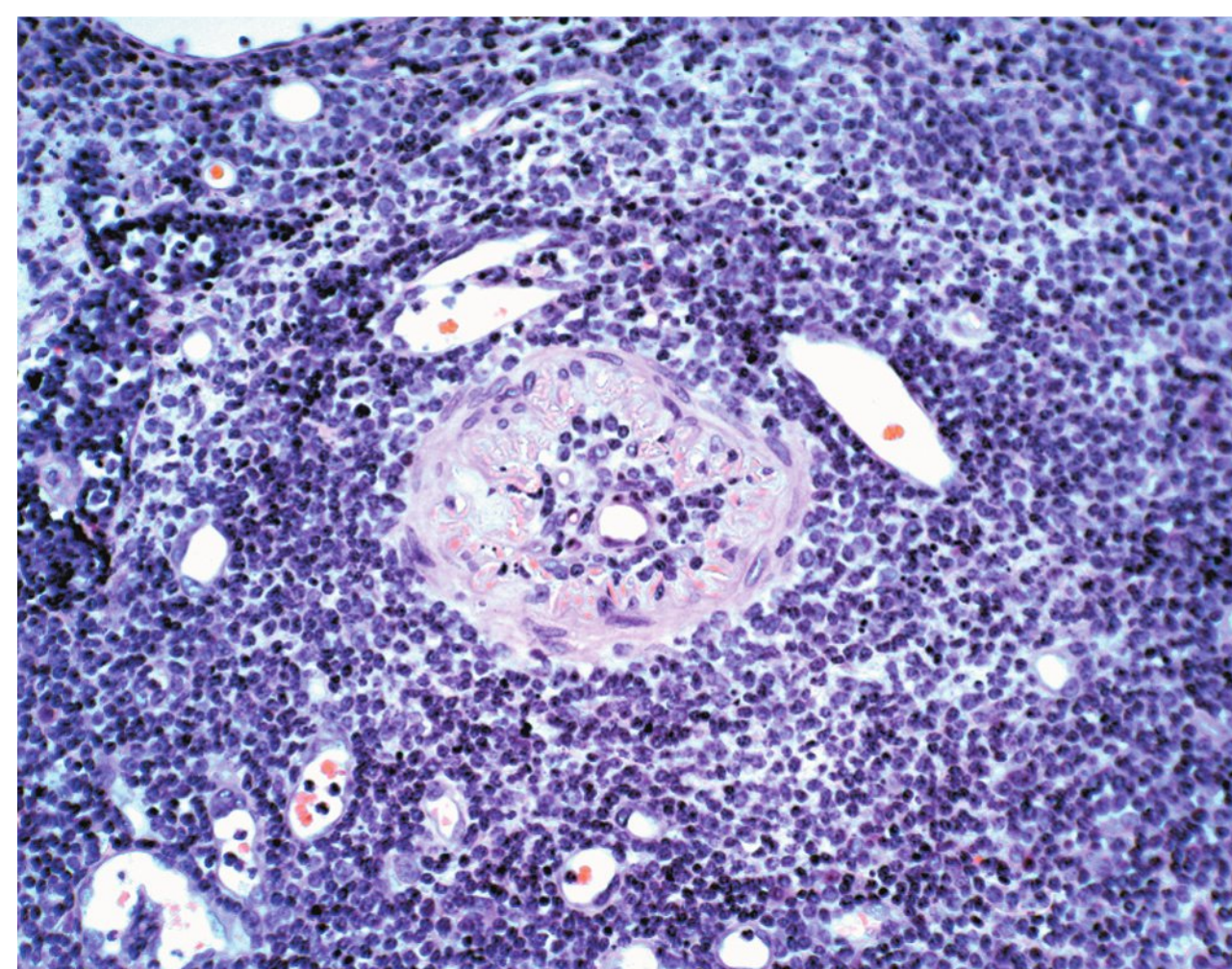


FIGURE 6.38.2 Case of extranodal NK- and/or T-cell lymphoma reveals angiocentric tumor cell infiltration. The artery in the center shows fibrinoid necrosis in the vessel wall. Hematoxylin and eosin, 60× magnification.

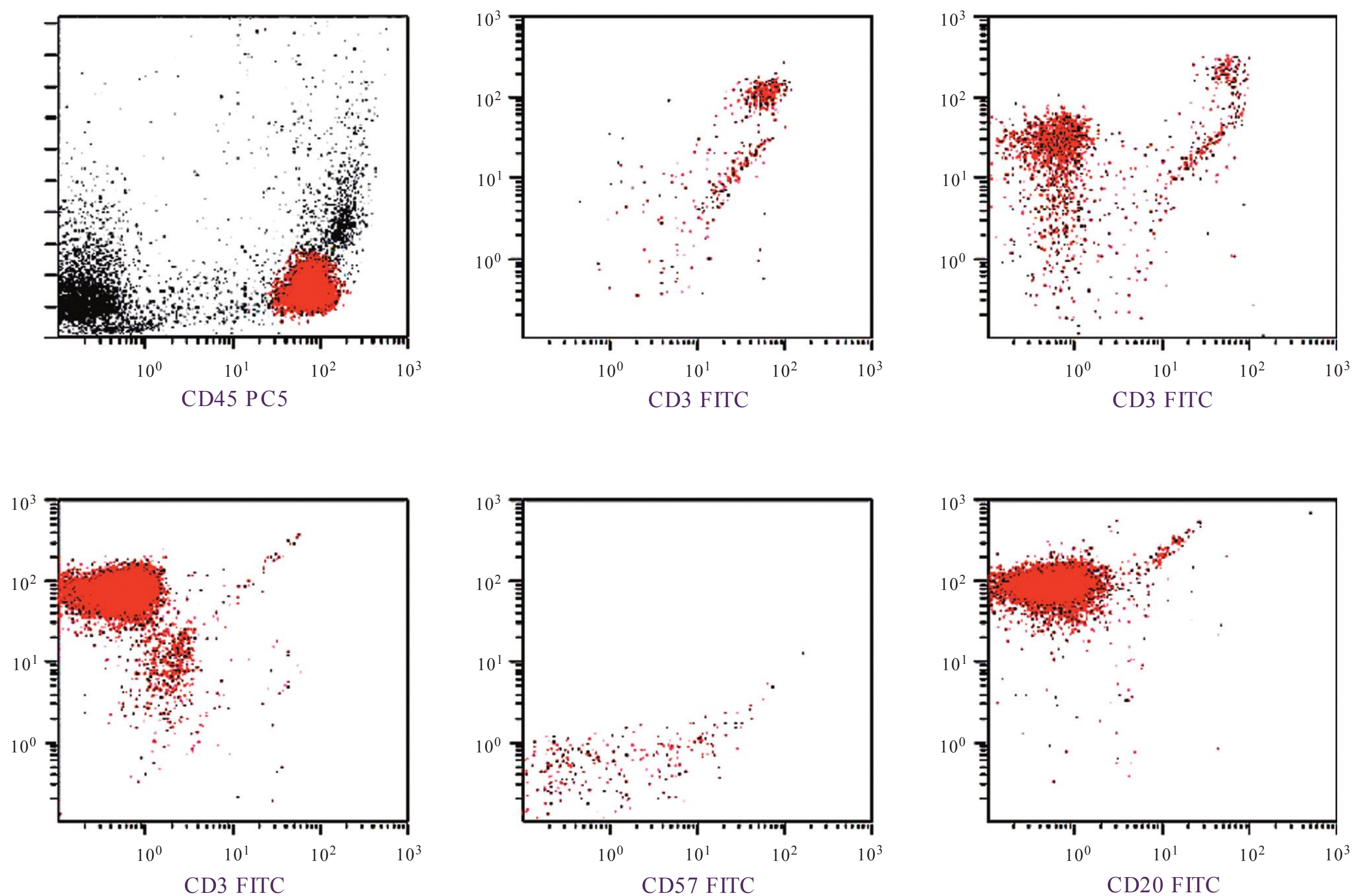


FIGURE 6.38.3 Flow cytometric histograms show positive reactions to CD2, CD8, and CD16-CD56, but negative reactions to CD3, CD4, CD20, and CD57. A side scatter versus CD45 plot is used for gating. SS, side scatter; PC5, phycoerythrin-cyanin 5; RD1, rhodamine; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

MOLECULAR GENETICS

Cytogenetic karyotyping of the tissue biopsy demonstrated a normal male karyotype of 46, XY. Germline configuration was detected with the T-cell receptor (TCR) gene probes and immunoglobulin (Ig) heavy-chain and light-chain gene probes.

DISCUSSION

This case illustrates the requirement of a high index of suspicion in the diagnosis of NK-cell lymphomas. First, the extensive necrosis and hemorrhages, frequently with superimposed infection in the angiocentric lymphoma, are frequently dismissed as an infectious process. Second, the identification of positive T-cell markers in the cellular infiltrate can be conceived as an inflammatory infiltration or a T-cell lymphoma, if T-cell subset study is performed.

It is particularly difficult to make the diagnosis of NK neoplasm in its early stage, because of the presence of a polymorphic cellular infiltration in the early stage of the disease (1–5). It is understandable, therefore, that the early literature frequently referred to this disease as indeterminate malignancy or polymorphic reticulosis.

The location in the midline of the head in the current case provides a clue to the possibility of an NK tumor, particularly nasal NK/T-cell lymphoma. Because of this particular location, early literature also referred to this tumor as malignant midline reticulosis, lethal midline granuloma, or midfacial destructive disease. However, these terms probably encompass a great variety of diseases, including lymphomatoid granulomatosis and Wegener granulomatosis (3,5). The similarity in morphology among these diseases is another cause of difficulty in the diagnosis of NK-cell neoplasms.

The presentation of tissue necrosis is mainly due to the angioinvasive and angiodestructive behavior of the tumor cells; therefore, it is often presented as zonal necrosis. Because of this histologic pattern, this tumor was designated by the Revised European American classification of lymphoid neoplasms as angiocentric T-cell lymphoma. However, angiocentric lesions can also be detected in other lymphomas, so the World Health Organization (WHO) classification changed it to extranodal NK/T-cell lymphoma, nasal type. Nevertheless, an angiocentric pattern is not invariably present in all NK-cell neoplasms; thus, the necrotic lesion is considered to be induced by more than one factor. Obviously, the release of cytotoxic proteins, T-cell intracellular antigen-1 (TIA-1), granzyme B, and perforin from the NK cells is another contributing factor to

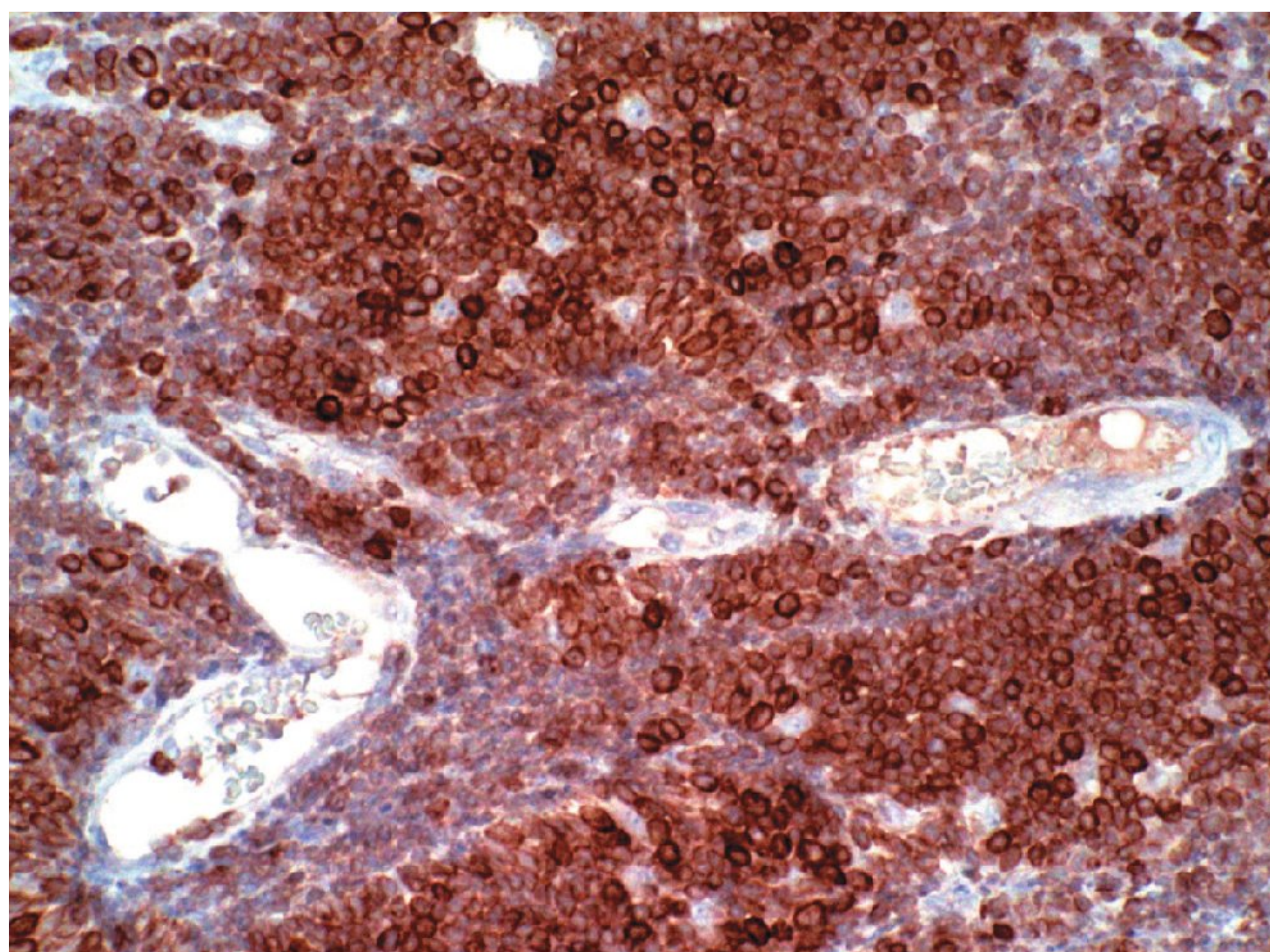


FIGURE 6.38.4 Case of extranodal NK/T-cell lymphoma reveals angiocentric tumor cell infiltration with positive CD3 staining. Immunoperoxidase, 60× magnification.

the necrotic lesion (6). Finally, the increased expression of the chemokines Ip-10 and Mig in the tumor cells, which is probably induced by Epstein–Barr virus (EBV) infection, is an additional contributing factor (7).

The predisposing factor of NK-cell tumor in this patient is not clear, but there is a strong association of nasal NK/T-cell lymphoma with EBV, which is present in >95% of patients, irrespective of the ethnic origin (3,5,8,9). On the contrary, nasal lymphomas of T-cell or B-cell origin have only a weak association with EBV; this fact further strengthens the argument for the etiological role of EBV in NK-cell tumors. Monoclonal EBV genome rearrangement is detected in NK tumor cells and is one parameter to verify the neoplastic nature of this lesion.

Similar to nasopharyngeal carcinoma and Hodgkin lymphoma, nasal T-/NK-cell lymphoma has the type II EBV latency pattern, as manifested by the presence of EBV

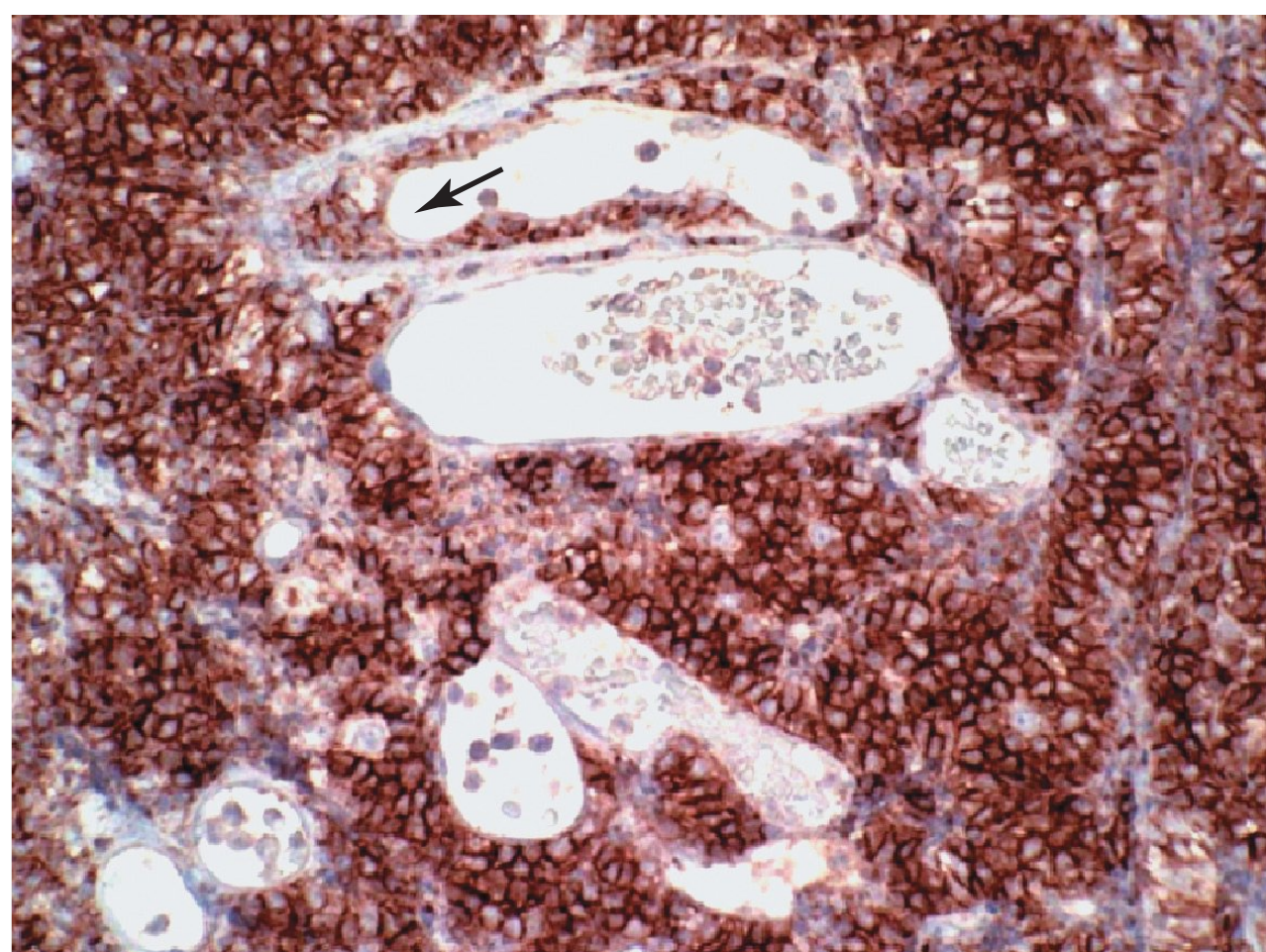


FIGURE 6.38.5 The same case as Figure 6.38.4 shows positive CD56 staining. The positive staining highlights the invasive pattern of the tumor cells in the blood vessel wall (arrow). Immunoperoxidase, 60× magnification.

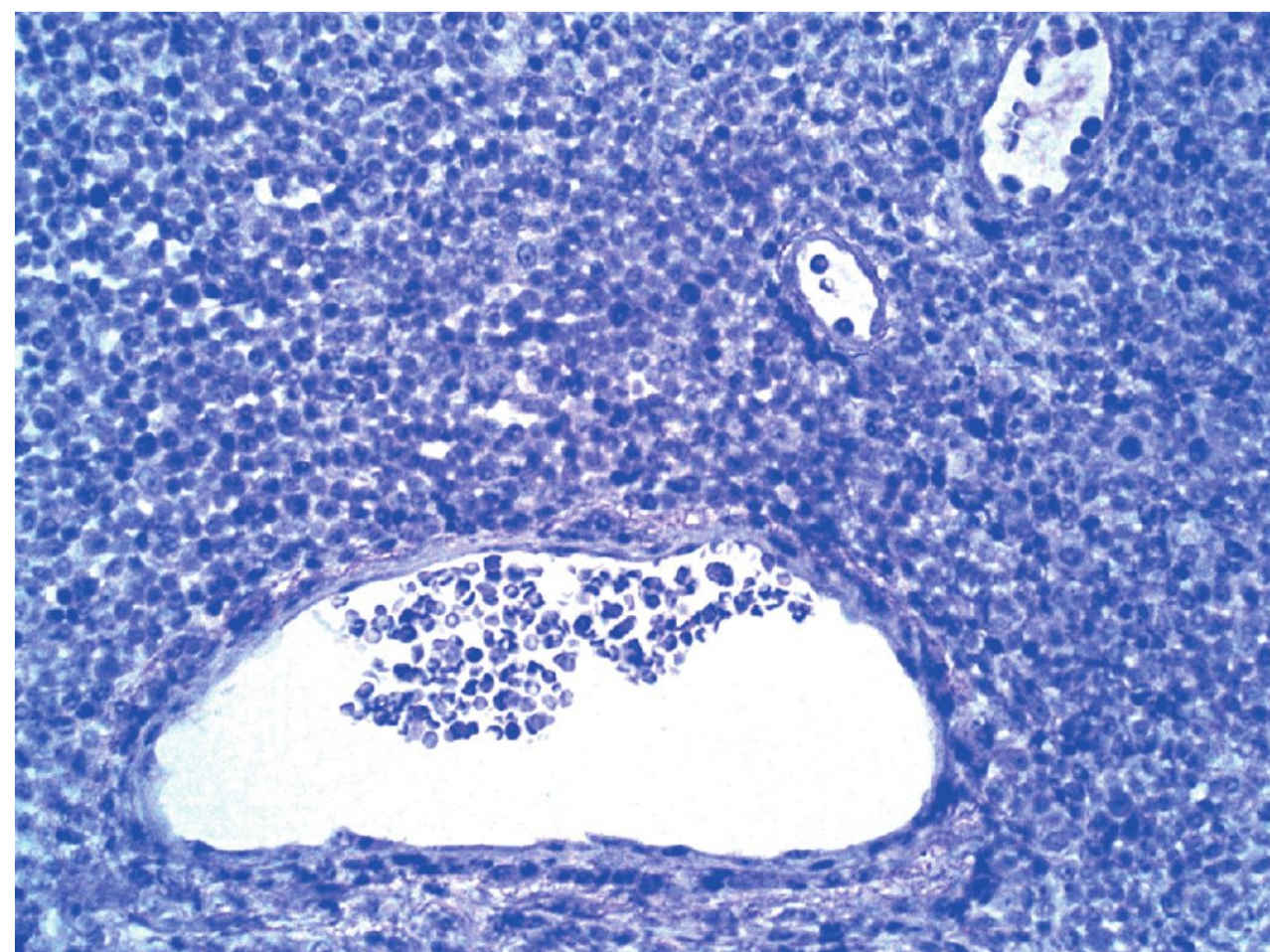


FIGURE 6.38.6 The same case as Figure 6.38.4 shows negative CD57 staining. Immunoperoxidase, 60× magnification.

nuclear antigen and the latent membrane protein 1 and 2 (3,5). Wegener granulomatosis is EBV negative, whereas lymphomatoid granulomatosis is an EBV-positive B-cell lymphoma (3,5). Therefore, the study of EBV is helpful in the differential diagnosis.

There are many different forms of NK-cell neoplasm reported. The nomenclature is confusing and overlapping. The current WHO classification of NK-cell tumor is probably not comprehensive, but it provides the baseline for a unifying designation. The 2001 WHO scheme enlists three NK-cell tumors: blastic NK-cell lymphoma, extranodal NK/T-cell lymphoma, nasal type, and aggressive NK-cell leukemia (10). However, the blastic NK-cell lymphoma was reclassified as blastic plasmacytoid dendritic cell neoplasm (see Case 15) in the 2008 WHO classification (11). Chronic lymphoproliferative disorder of NK cells is added to this new version (12). In addition, natural killer cell lymphoblastic leukemia/lymphoma is considered a provisional entity in the category of ambiguous leukemias.

The term CD3– NK leukemia or NK-LGL leukemia is in the category of aggressive NK-cell leukemia (13). The indolent NK large granular lymphocyte (NK-LGL) leukemia or chronic NK-cell lymphocytosis is equivalent to the chronic lymphoproliferative disorder of NK cells (12). In the earlier literature, there were reports on two precursor NK-cell neoplasms: myeloid/NK-cell precursor acute leukemia and myeloid/NK-cell acute leukemia (14–18); these tumors are now considered probable acute myeloid leukemia with expression of NK markers.

Morphology

NK cells assume the morphology of LGLs, which show abundant pale or transparent cytoplasm with azurophilic granules and a round or kidney-shaped nucleus with a mature chromatin pattern (Fig. 6.38.7). In chronic lymphoproliferative disorder of NK cells, the LGLs appear normal and are indistinguishable from those seen in T-cell large granular lymphocytic leukemia (T-LGL leukemia) (12). The cytoplasmic granules of NK cells can be demonstrated in

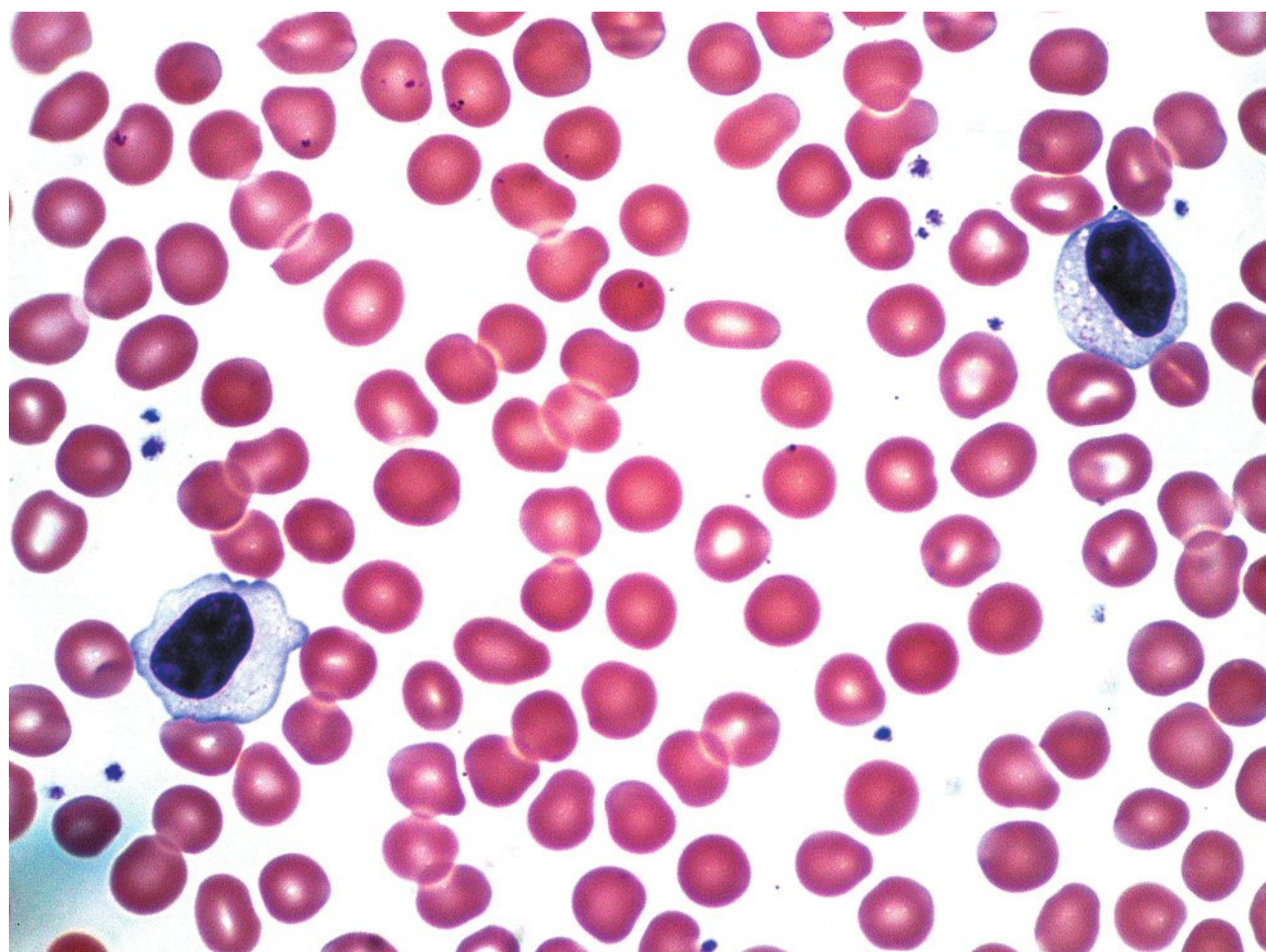


FIGURE 6.38.7 Peripheral blood smear shows two normal LGLs with delicate cytoplasmic granules. Wright-Giemsa, 150× magnification.

peripheral blood smears and bone marrow aspirates, but not in tissue sections. Therefore, it is important to make touch imprints from lymph node, spleen (Fig. 6.38.8), and other solid tissue masses to look for cytoplasmic granules before the specimen is fixed.

The original criteria for NK/T-LGL leukemia are the persistent presence of 2,000 LGL/ μ L or >40% of the lymphocyte fraction for at least 6 months, and that all possible causes for reactive lymphocytosis are excluded (19,20). The more recent cutoff for the diagnosis of T-LGL leukemia is 500 or 520/ μ L of CD3+/CD57+ cells (21,22). The new cutoff for NK-LGL leukemia is 600/ μ L (14). The 2008 WHO classification defines chronic lymphoproliferative disorder of NK cells as a persistent (6 months) increase in peripheral blood NK cells (usually 2,000/ μ L) without a clearly identified cause (12).

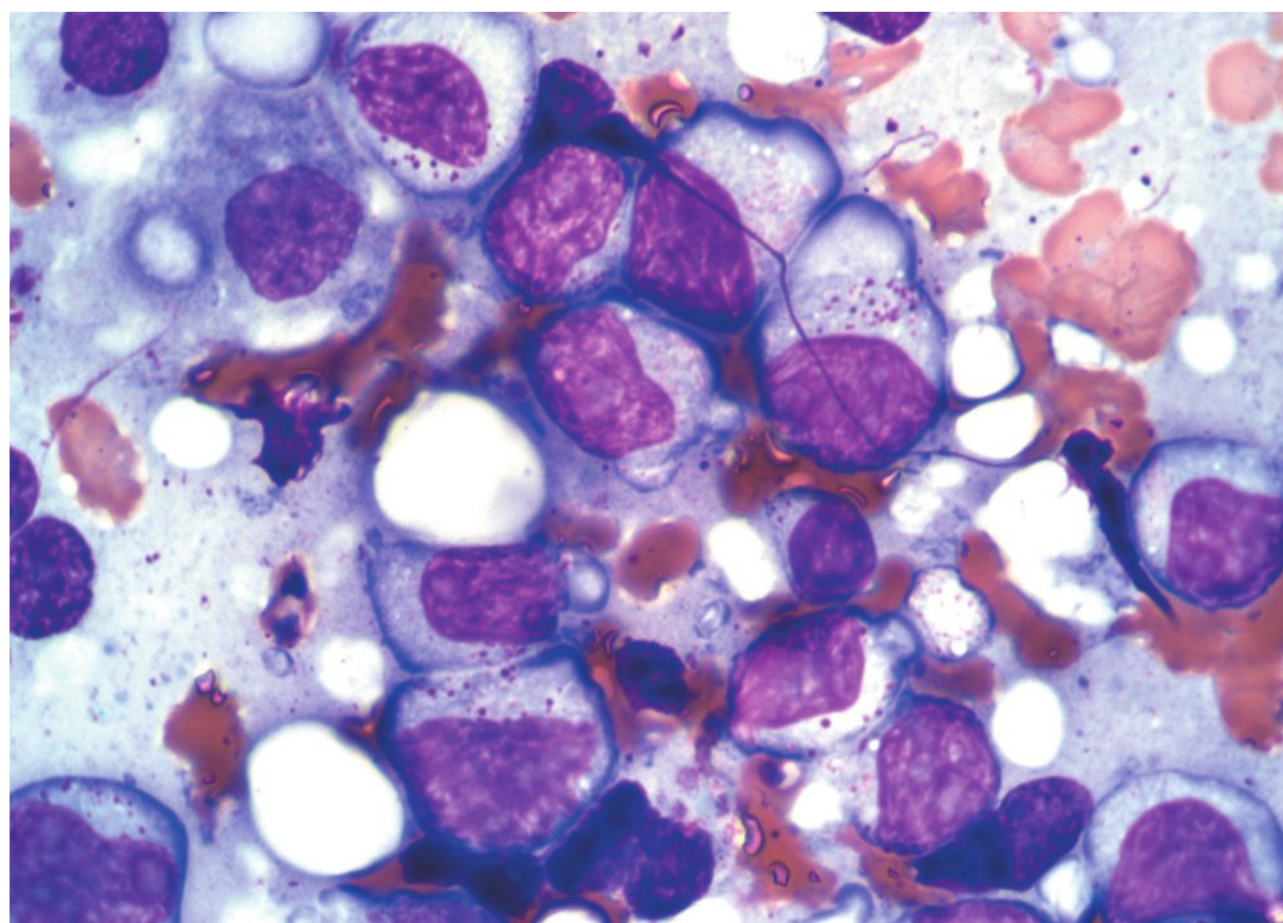


FIGURE 6.38.8 Spleen imprint from a case of aggressive NK-cell lymphoma/leukemia shows a cluster of leukemic cells with cytoplasmic granules. Wright-Giemsa, 100× magnification.

Extranodal NK/T-cell lymphomas have a broad cytologic spectrum with variable cell size (1,23). Therefore, the only diagnostic feature is the presence of cytoplasmic granules, which can be identified only in the touch preparation of the tumor. In the early stage, the lymphoma cells are intermixed with reactive inflammatory cells, including small lymphocytes, histiocytes, neutrophils, eosinophils, and plasma cells (1–5). Therefore, these cases would be easily mistaken as chronic inflammatory diseases. In the late stage, a full-blown feature of pleomorphic tumor cells with irregular nuclei and granular chromatin may finally prevail (30).

The histologic pattern is more helpful than cytology in the diagnosis of this type of NK tumor. Regardless of the location, the pathognomonic features are angioinvasion, angiodestruction, and zonal necrosis in the majority of cases (Fig. 6.38.9) (1,4,24–29).

In recent years, primary intestinal NK-like T-cell lymphoma has emerged as a distinct entity (30,31). It is characterized by the presence of monomorphic small-to-medium tumor cells, absence of villous atrophy and crypt hyperplasia, and no association with enteropathy and celiac disease. This entity should be distinguished from intestinal NK-cell lymphoma and enteropathy-associated T-cell lymphoma by immunophenotyping and karyotyping.

The tumor cells of the aggressive NK-cell lymphoma/leukemia are usually highly pleomorphic and often contain irregular cytoplasmic azurophilic granules, which are more prominent than those seen in normal LGLs (Fig. 6.38.10) (26,29–36). However, the leukemic cells may also show no obvious atypia, which makes the diagnosis difficult (13). In addition, the leukemic cells may not appear in an early stage of clinical course, which further delays the diagnosis (33). In the bone marrow, the infiltration pattern is also variable; it can be massive, focal, or subtle (13). Angioinvasion and angiodestruction can be observed in this tumor (4). Hemophagocytosis is also a common feature (1,4,24,29). In the spleen, this leukemia shows predominantly red pulp involvement (37).

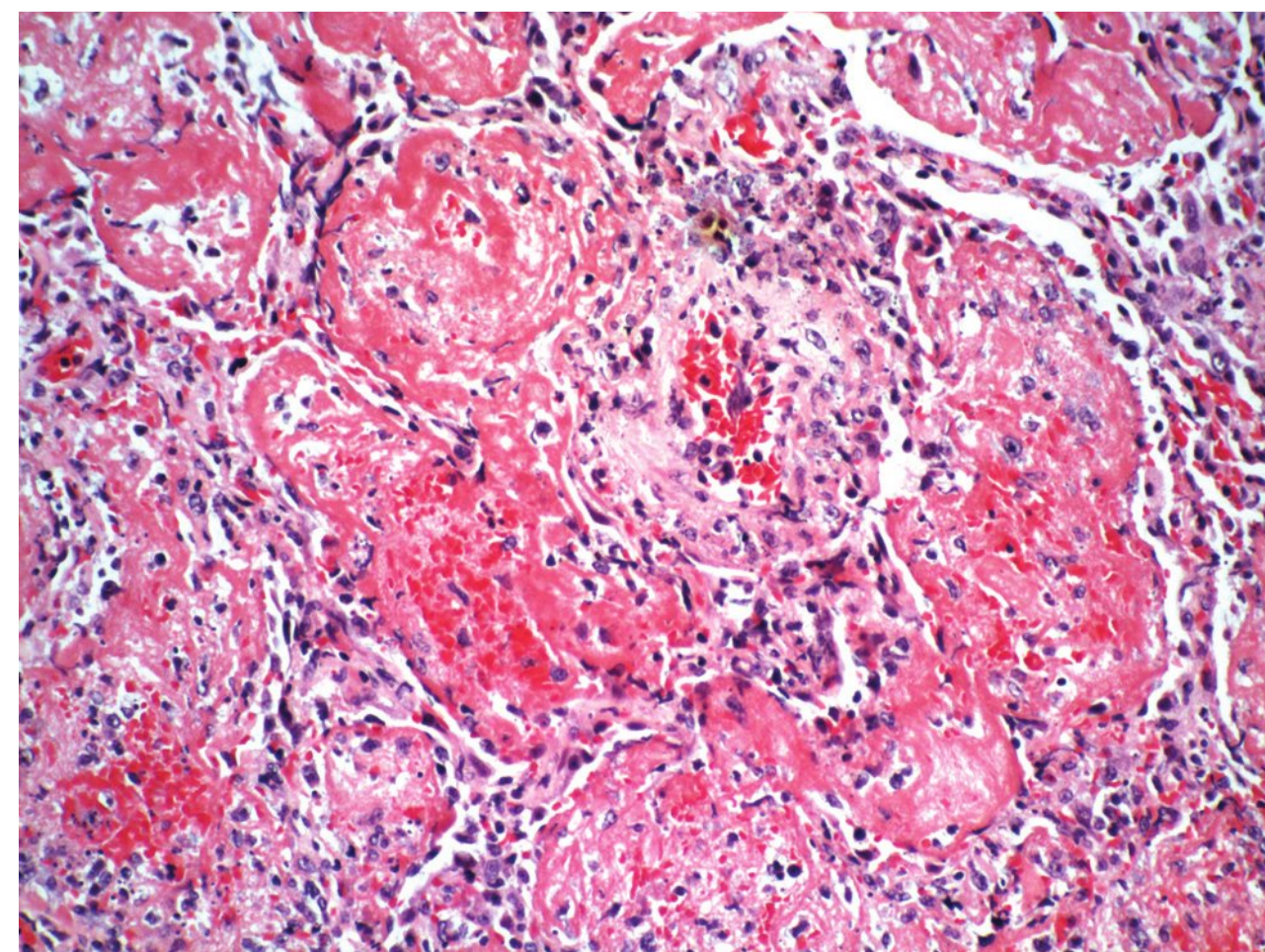


FIGURE 6.38.9 Lung biopsy shows tumor cell infiltration of the blood vessel wall and intra-alveolar hemorrhages and fibrinous exudates. Hematoxylin and eosin, 40× magnification.

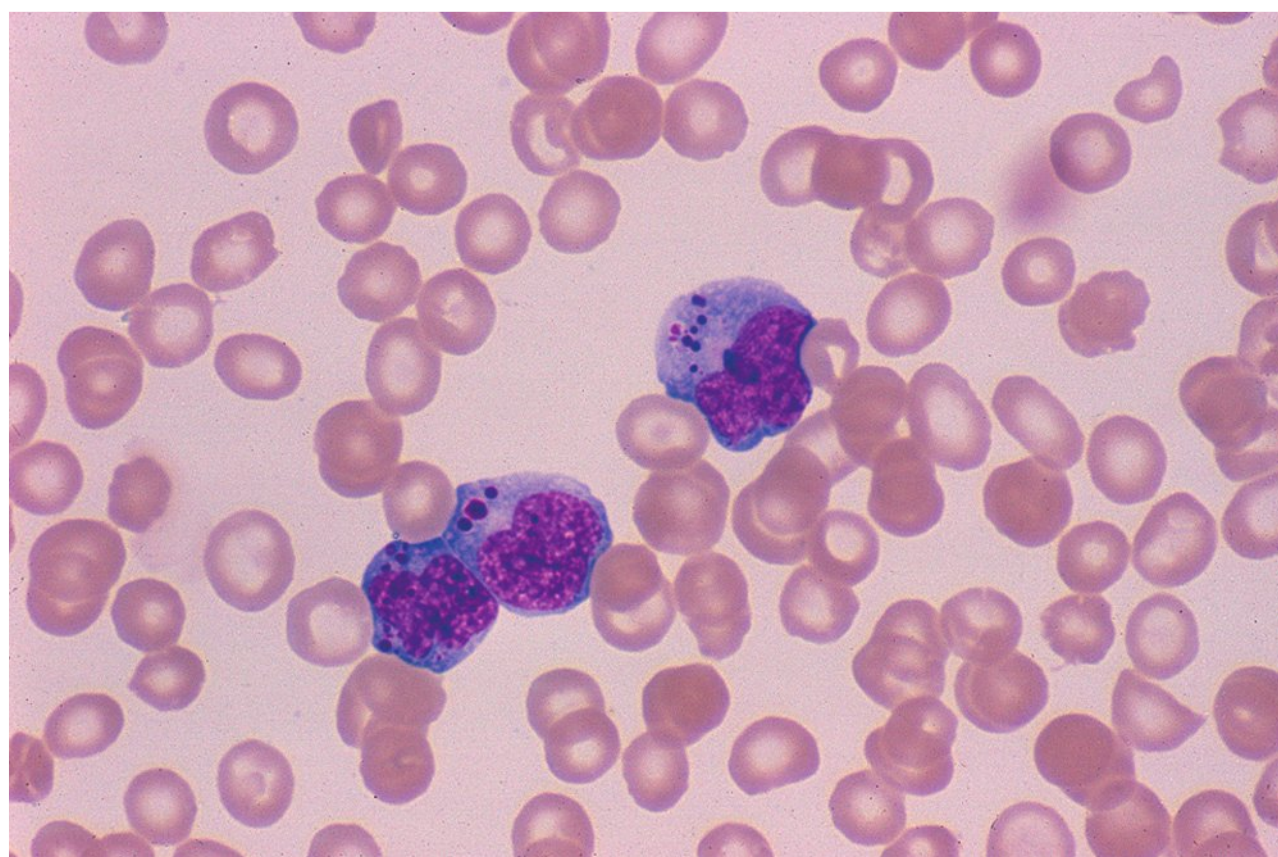


FIGURE 6.38.10 Peripheral blood smear from a case of aggressive NK-cell lymphoma/leukemia shows three tumor cells with prominent cytoplasmic granules. Wright-Giemsa, 150× magnification.

Immunophenotype

Chronic lymphoproliferative disorder of NK cells and T-LGL leukemia can be distinguished only by immunophenotyping (38). The usual phenotype of the former is CD3[−], CD4[−], CD8[−], CD16⁺, CD56⁺, and CD57[−] and that of the latter is CD3⁺, CD4[−], CD8⁺, CD16⁺, CD56[−], and CD57⁺ (3,22,39). However, NK cells can show CD57 and CD8, whereas T-LGL (NK-like T cells) may express CD56. The major difference between these two entities is the presence or the absence of surface CD3; NK cell is surface CD3 negative but cytoplasmic CD3 (CD3) positive. Therefore, in immunochemical stains, NK-cell tumors always express CD3 and cause some confusion for the classification.

CD3 and TCR form a complex on all T lymphocytes. Accordingly, T cells and NK-like T cells express TCR proteins (TCR_{ab} or TCR_{gd}) that are associated with TCR gene rearrangements. NK cells, in contrast, show neither TCR protein

nor TCR gene rearrangements. In addition, CD2, CD11b, and CD11c are consistently present on NK cells, but the expression of CD5 and CD7 is variable. However, one report emphasized the absence of CD5 in NK-cell lymphomas (40). Another important characteristic of NK and NK-like T-cells is that both kinds of cells contain cytotoxic proteins (TIA-1, granzyme B, and perforin) (3,41). The differences between NK and NK-like T cells are summarized in Table 6.38.1.

Most cases of extranodal NK/T-cell lymphoma, particularly those found in Asian countries, have an NK-cell phenotype and are CD56 positive (3,5,9,24,26). Virtually all of these cases are EBV positive. In immunochemical staining, the routine T-cell markers, such as CD43 and CD45RO, in addition to cytoplasmic CD3 (CD3), are all positive. These results frequently cause confusion as to whether these tumors represent NK-like T-cell lymphoma or NK lymphoma. Cutaneous CD56⁺, NK/T-cell lymphomas frequently express cell adhesion molecules (CD2, CD11a, and CD49d) and their ligands (CD58, CD54, and CD106), which are more frequently associated with an angi-destructive histopathologic pattern than those without the expression of these molecules (42).

The primary intestinal NK-like T-cell lymphoma has a characteristic immunophenotype of CD3[−], cytoplasmic CD3⁺, CD4[−], CD8⁺, CD5[−], CD7⁺, CD16[−], CD56⁺, CD57[−], CD103⁺, TIA-1⁺, granzyme B⁺ and bF1⁺ (30,31). This tumor shows TCR gene rearrangement and variable EBV-positivity (30,31). The intestinal NK-cell lymphoma has a similar immunophenotype with consistent EBV infection, but absence of TCR gene rearrangement and no expression of TCR proteins (TCRd, bF1). The enteropathy-associated T-cell lymphoma has two different immunophenotypes (43). The pleomorphic form shows CD3⁺, CD5[−], CD7⁺, CD8[±], CD4[−], CD103⁺, TCRb[±] and cytotoxic proteins⁺. The monomorphic form has an immunophenotype of CD3⁺, CD4[−], CD8⁺, CD56⁺, and TCRb⁺.

The immunophenotype of aggressive NK-cell lymphoma/leukemia is similar to that of extranodal NK/T-cell

TABLE 6.38.1

Differences between NK Cells and NK-Like T Cells

	NK cell	NK-Like T cell
sCD3	Negative	Positive
cCD3	Positive	Positive
CD4	Negative	Negative
CD8	Negative/positive	Positive
CD16	Positive/negative	Positive/negative
CD56	Positive	Negative
CD57	Negative	Positive
Cytotoxic proteins	Positive	Positive
NK-cell receptors	Positive	Negative/positive
TCR _{ab} /TCR _{gd} proteins	Negative	Positive
TCR gene rearrangement	Negative	Positive

NK, natural killer; TCR, T-cell receptor.



lymphoma, and EBV is almost always present in the tumor cells (3,14,26,29). The distinction between these two types of tumors is their clinical manifestations. A comprehensive monoclonal antibody panel presented by Suzuki et al includes CD1–, CD2+, CD3–,cytoplasmic CD3±, CD4–, CD5–, CD7+, CD8±, CD10–, CD11b±, CD13–, CD16+, CD19–, CD20–, CD25–, CD33–, CD34–, CD38+, CD56+, CD57±, CD122+, HLA-DR+, and TCR– (44).

In addition to the above markers, there are two new systems that are involved with T cells and NK cells. The first one is the chemokine receptors, which control lymphocyte trafficking (45). The CC-chemokine receptor R7 (CCR7) can be used to divide memory T cells into two distinct subsets: the central memory cells (CCR7+) and the effector memory cells (CCR7–). Both NK- and T-cell lymphoproliferative diseases of granular lymphocytes (LDGL) express the CCR7– phenotype (45).

Another one is NK receptors (NKR), which can be divided into killer Ig-like receptors (KIR), leukocyte Ig-like receptors and/or Ig-like transcripts, killer lectin-like receptor (KLR), natural cytotoxicity receptors (NCR), coreceptors, and other receptors (45,46). The two major NKRs are KIR, which recognizes classical major histocompatibility complex (MHC) class I molecules; and C-type KLR, which recognizes nonclassical MHC-related molecules (60). Both NK- and T-LDGL show restricted NKR subsets, and CD94 (a KLR marker) is most commonly expressed. Another study demonstrated dysregulated NKR expression in patients with the NK type of lymphoproliferative disease (47).

Based on the expression of two KLR markers (CD94 and CD161) and CD56, NK cells can be divided into three developmental stages (48). The pre-NK cells express CD161; immature NK cells express CD161 and CD56; and mature NK cells express CD161, CD56, and CD94. A recent review article further divides NK cells into five developmental stages (Table 6.38.2) (49).

The study by Mori et al. (48) shows that blastic NK-cell lymphoma/leukemia (currently classified as blastic plasmacytoid dendritic cell neoplasm) has an immunophenotype of CD56+, CD94–, CD161–, but aggressive NK-cell

leukemia/lymphoma, nasal NK/T-cell lymphoma, nasal type, and chronic lymphoproliferative disorder of NK cells express both CD56 and CD94 (48).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry plays an important role in distinguishing between NK-cell and NK-like T-cell neoplasms because it can differentiate surface CD3 and cytoplasmic CD3 staining in the tumor cells. In addition, flow cytometry may be used to demonstrate the chemokine and NKRs. In contrast, cytotoxic proteins can be detected only by immunohistochemical stains.

Molecular Genetics

Cells in NK tumors are characterized by the absence of the TCR gene (both b- and g-chains) and Ig gene rearrangements. However, the clonality of NK-cell tumors can be determined by X-linked DNA analysis, which is only applicable to female patients (50). If the tumor contains EBV DNA, the clonality can also be identified by EBV genome rearrangement (51).

The most common genetic abnormalities in NK-cell tumors are loss or gain of genetic materials. Deletion of 6q has the highest frequency (1). Other common abnormalities include del(11q), del(13q), del(17q), i(1q), i(6q), and 3+xp (52,53). However, the general consensus is that numerical genetic abnormalities usually represent secondary changes. In recent years, several case reports showed chromosome translocations, mainly involving 8p23, including der(8)t(8;8)(p23;q13) in a case of aggressive NK-cell leukemia/lymphoma (33), der(8)t(8;17)(p23;q24) in a case of nasal type NK-/T cell lymphoma (54), and der(8)t(1;8)(q10;p23) in a case of aggressive NK-cell leukemia (54). An add(8)(q23) abnormality was reported in a case of aggressive NK-cell lymphoma (54), and a nasal NK-/T-cell lymphoma (55). Therefore, translocation involving 8p23 appears to be a nonrandom chromosomal change in NK-cell tumors, especially aggressive NK-cell leukemia and extranodal NK-/T-cell lymphoma, nasal type.

TABLE 6.38.2

Developmental Stages of Human NK Cells

	Pro-NK	Pre-NK	I-NK	CD56 ^{bright} NK	CD56 ^{dim} NK
CD34	+	+	–	ND	ND
CD45RA	+	+	ND	ND	ND
CD10	+	–	ND	ND	ND
CD117	–	+	+	±	–
CD161	–	±	+	ND	ND
NKp46	ND	ND	–	+	+
CD94/NKG2A	ND	ND	–	+	±
CD16	ND	ND	ND	–	+
KLR	ND	ND	ND	±	+

I-NK, intermediate NK; KLR, Killer lectin-like receptor; ND, no data

A report on the loss of material on 16p and 17p may represent the loss of p53 in the NK-cell tumor. In one study of 28 cases of nasal T-/NK-cell lymphomas, 86% had overexpression of p53 (25). Molecular studies have also revealed inactivation of multiple tumor suppressor genes, including p10NK4A, p15NK4B, p14ARF, TP53, and Rb (53).

Several studies showed the overexpression of Fas molecule (CD95) and Fas ligand (CD95L) in nasal T-/NK-cell lymphoma (56). In addition, high levels of soluble serum CD95L have also been found in NK-cell lymphoma/leukemia in recent studies. The interaction between CD95 and CD95L may facilitate local tissue invasion, distant metastasis, systemic tissue damage, and immune evasion and is presumably the contributing factor to the marked aggressive behavior of these tumors.

Gene expression profiling study of extranodal NK/T-cell lymphoma, nasal type, reveals perturbation of angiogenic pathways and deregulation of the tumor suppressor HACE1 in the frequently deleted 6q21 region (57). In the same region, three known genes PRDM1, ATG5, and AIM1 are also downregulated. These genomic alterations may have contributed to the pathogenesis of NK-cell malignancies (58). Of particular interest is the finding of aberrant overexpression of microRNA in NK-cell lymphoma/leukemia (59). This study has found that miR-21 and miR-155 are overexpressed in NK-cell neoplasms, probably secondary to EBV infection. As a result, AKT signaling is affected due to the repression of PTEN and SHIP1 by these two microRNAs, respectively, leading to pathogenesis of NK-cell neoplasms (59).

The salient features for laboratory diagnosis of NK-cell lymphoma or leukemia are summarized in Table 6.38.3.

Clinical Manifestations

Chronic lymphoproliferative disorder of NK cells is defined by the 2008 WHO classification as the presence of 2,000/mL NK cells for more than 6 months without a clearly identified cause (12). Most of the reported cases may represent reactive lymphocytosis, but the minority of cases may show slow progress with organ involvement. The clinical presentation may be similar to that of T-LGL leukemia. Rheumatoid arthritis, pure red cell aplasia, vasculitis, and cyclic neutropenia are seen in rare cases (22). Neutropenia, if present, is moderate. Splenomegaly is the most frequent physical finding, but it is not as frequently seen as it is in T-LGL leukemia. Hepatomegaly and lymphadenopathy are rare. Recurrent bacterial infections are the major clinical presentation in cases with severe neutropenia. Substantial increases in NK cells have been observed in patients with other lymphomas, leukemias, solid tumors, splenectomy, neuropathy, autoimmune disorders, or myelodysplastic syndrome (12,60,61). Therefore, these conditions should be included in the differential diagnosis.

Extranodal NK/T-cell lymphoma, nasal type, is the most common primary nasal lymphoma, especially in cases where the paranasal sinus is not involved. However, the involvement of nasopharynx and paranasal sinuses is seen in 37% to 50% of patients (26). The early clinical presentation is nasal obstruction, nasal discharge, and epistaxis. It finally progresses to a destructive nasal or midline facial tumor, with palatal destruction, orbital swelling, and

TABLE 6.38.3

Salient Features for Laboratory Diagnosis of NK-Cell Lymphomas

1. Demonstration of azurophilic cytoplasmic granules in tumor cells by Wright–Giemsa stain on smear of peripheral blood or bone marrow, or tissue touch preparation
2. Expression of one or two NK markers (CD56, CD57, CD16)
3. Absence of surface CD3 but presence of cytoplasmic CD3 (CD3)
4. Presence of cytotoxic proteins: Perforin, granzyme B, and TIA-1
5. Expression of some myeloid antigens (CD11b, CD11c)
6. Absence of B-cell antigens and other myeloid antigens
7. No expression of TCR_{ab} or TCR_{gd} proteins
8. No TCR gene rearrangements
9. Frequent demonstration of EBV genomes in tumor cells or EBV antibodies

EBV, Epstein–Barr virus; NK, natural killer; TCR, T-cell receptor; TIA, T-cell intracellular antigen.

edema. Although this tumor is usually localized in presentation, it may disseminate to distant sites, such as the skin, gastrointestinal tract, liver, lung, and testis (2,24,26). When an NK tumor is present as a primary tumor in these sites without nasal involvement, it is called extranasal NK/T-cell lymphoma (Fig. 6.38.11). The extranasal tumor usually carries a worse prognosis than the primary nasal tumor.

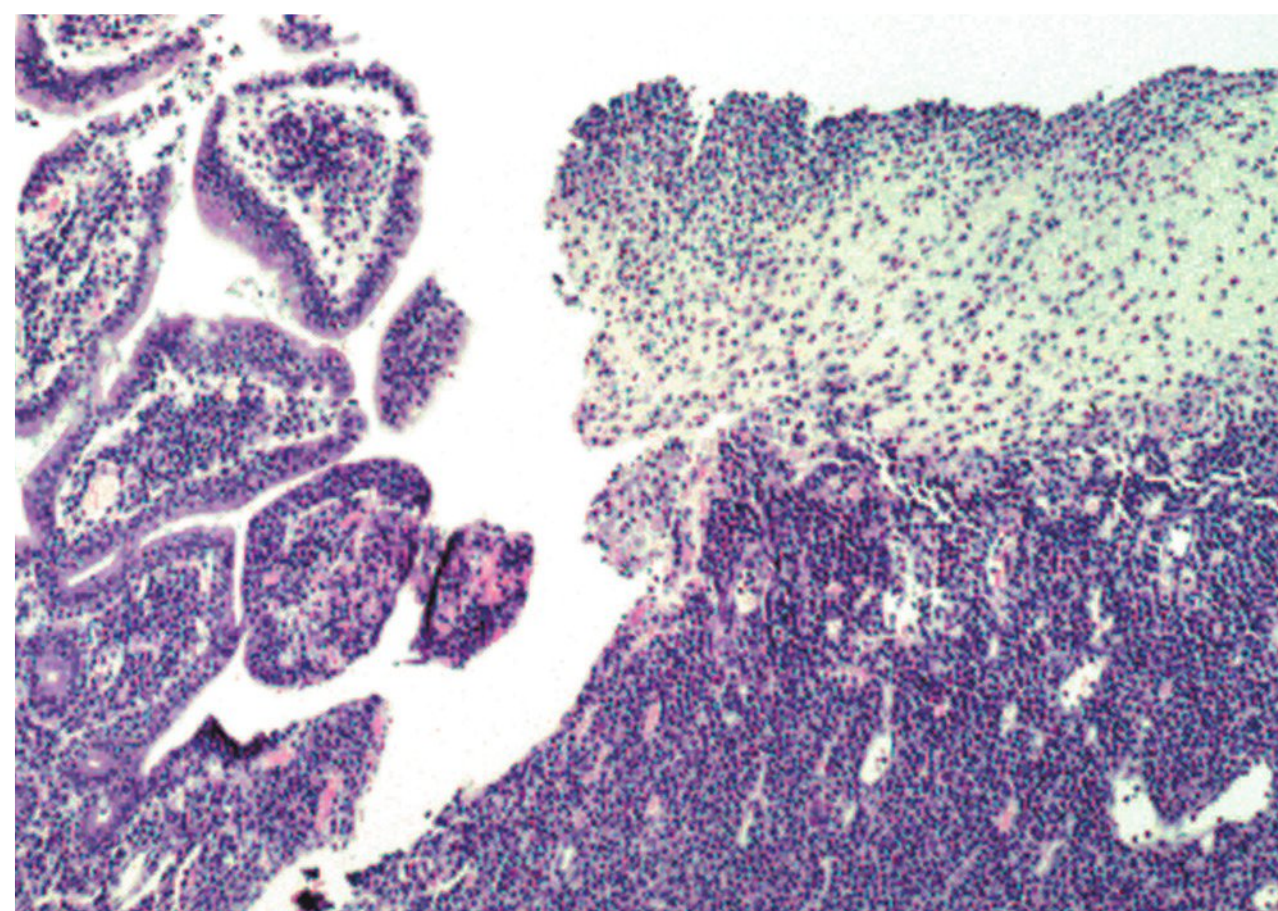


FIGURE 6.38.11 Case of extranodal NK- and/or T-cell lymphoma involving the ileum shows extensive tumor cell infiltration and destruction of normal architecture. The ulcerative mucosal surface is covered with fibrinopurulent exudates. Hematoxylin and eosin, 20× magnification.

The aggressive NK-cell lymphoma or leukemia is usually presented with systemic dissemination of the disease with bone marrow and late peripheral blood involvement (26,28,32,36). Constitutional symptoms including fever, coagulopathy, bleeding tendency, hemophagocytic syndrome, and multiorgan failure are common. Most patients have hepatosplenomegaly, but lymphadenopathy and skin lesions may vary from patient to patient. Most patients are young and succumb within weeks to months after the diagnosis.

REFERENCES

- Jaffe ES, Chan JKC, Su JJ, et al. Report of the workshop on nasal and related extranodal angiocentric T/natural killer cell lymphomas: definition, differential diagnosis and epidemiology. *Am J Surg Pathol*. 1996;20:103–111.
- Jaffe ES, Krenacs L, Raffeld M. Classification of cytotoxic T-cell and natural killer cell lymphomas. *Semin Hematol*. 2003;40:175–184.
- Kinney MC. The role of morphologic features, phenotype, genotype, and anatomic site in defining extranodal T-cell or NK-cell neoplasms. *Am J Clin Pathol*. 1999;111(suppl 1):S104–S118.
- Kwong YL, Chan ACL, Liang RHS. Natural killer cell lymphoma/leukemia: pathology and treatment. *Hematol Oncol*. 1997;15:71–79.
- Burke JS. Waldeyer's ring, sinusoidal region, salivary gland, thyroid gland, central nervous system, and other extranodal lymphomas and lymphoid hyperplasias. In: Knowles DM, ed. *Neoplastic Hematology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1351–1389.
- Ng CS, Lo STH, Chan JKC, et al. CD56+ putative natural killer cell lymphomas: production of cytolytic effectors and related proteins mediating tumor cell apoptosis? *Hum Pathol*. 1997;90:4099–4105.
- Teruya-Feldstein J, Jaffe ES, Burd PR, et al. The role of Mig, the monokine induced by interferon- γ and IP-10, the interferon- γ -inducible protein-10, in tissue necrosis and vascular damage associated with Epstein-Barr virus-positive lymphoproliferative disease. *Blood*. 1997;90:4099–4195.
- Chan JKC, Jaffe ES, Ralfkiaer E. Extranodal NK/T-cell lymphoma, nasal type. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:204–207.
- Yachie A, Kanegane H, Kasahara Y. Epstein-Barr virus-associated T/natural killer cell lymphoproliferative diseases. *Semin Hematol*. 2003;40:124–132.
- Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001.
- Facchetti F, Jones DM, Petrella T. Blastic plasmacytoid dendritic cell neoplasm. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:145–147.
- Willamor N, Morice WG, Foucar K. Chronic lymphoproliferative disorders of NK cells. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:274–275.
- Chan JKC, Jaffe ES, Ralfkiaer E, et al. Aggressive NK-cell leukaemia. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:276–277.
- Oshimi K. Leukemia and lymphoma of natural killer lineage cells. *Int J Hematol*. 2003;78:18–23.
- Suzuki R, Nakamura S. Malignancies of natural killer cell precursor: myeloid/NK cell precursor acute leukemia and blastic NK cell lymphoma/leukemia. *Leuk Res*. 1999;23:615–624.
- Nagai M, Bando S, Tasaka T, et al. Secondary myeloid/natural killer cell precursor acute leukemia following essential thrombocythemia. *Hum Pathol*. 1999;30:868–871.
- Sun T, Shayesteh P, Jaffrey I, et al. A hybrid form of myeloid/NK-cell acute leukemia and myeloid/NK-cell precursor acute leukemia. *Hum Pathol*. 2003;34:504–507.
- Liang X, Graham DK. Natural killer cell neoplasms. *Cancer*. 2008;112:1425–1436.
- Tefferi A, Li CY, Witzig TE, et al. Chronic natural killer cell lymphocytosis: a descriptive clinical study. *Blood*. 1994;84:2721–2725.
- Semenzato G, Zambello R, Starkebaum G, et al. The lymphoproliferative disease of granular lymphocytes: updated criteria for diagnosis. *Blood*. 1997;89:256–260.
- Greer JP, Kinney MC, Loughran TP Jr. T cell and NK cell lymphoproliferative disorders. *Hematology Am Soc Hematol*. 2001;259–281.
- Lamy T, Loughran TP Jr. Clinical features of large granular lymphocyte leukemia. *Semin Hematol*. 2003;40:185–195.
- Chan JKC, Quintanilla-Martinez L, Ferry JA, et al. Extranodal NK-T-cell lymphoma, nasal type. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:285–288.
- Chan JKC, Sin VC, Wong KF, et al. Nonnasal lymphoma expressing the natural killer cell marker CD56: a clinico-pathologic study of 49 cases of an uncommon aggressive neoplasm. *Blood*. 1997;89:4501–4513.
- Quintanilla-Martinez L, Franklin JL, Guerrero I, et al. Histological and immunophenotypic profile of nasal NK/T cell lymphomas from Peru: high prevalence of p53 overexpression. *Hum Pathol*. 1999;30:849–855.
- Cheung MMC, Chan JKC, Wong KF. Natural killer cell neoplasms: a distinctive group of highly aggressive lymphomas/leukemias. *Semin Hematol*. 2003;40:221–232.
- Jia H, Sun T. Extranodal NK/T-cell lymphoma mimicking cellulites. *Leuk Lymphoma*. 2004;45:1416–1470.
- Frank M, Sun T. An unusual case of peripheral T-cell lymphoma with CD56 positivity and angiocentric, angiodestructive morphology arising in the ileum. *Arch Pathol Lab Med*. 2005;129:527–530.
- Mori N, Yamashita Y, Tsuzuki T, et al. Lymphomatous features of aggressive NK cell leukaemia/lymphoma with massive necrosis, haemophagocytosis and EB virus infection. *Histopathology*. 2000;37:363–371.
- Muram-Zborovski T, Loeb D, Sun T. Primary intestinal intraepithelial natural killer-like T-cell lymphoma. *Arch Pathol Lab Med*. 2009;133:133–137.
- Chuang SS, Chang ST, Chuang WY, et al. NK-cell lineage predicts poor survival in primary intestinal NK-cell and T-cell lymphomas. *Am J Surg Pathol*. 2009;33:1230–1240.
- Imamura N, Kusunoki Y, Kawa-Ha K, et al. Aggressive natural killer cell leukaemia/lymphoma: report of 4 cases and review of the literature: possible existence of a new clinical entity originating from the third lineage of lymphoid cells. *Br J Haematol*. 1990;75:49–59.
- Sun T, Brody J, Susin M, et al. Aggressive natural killer cell lymphoma/leukemia: a recent recognized clinicopathologic entity. *Am J Surg Pathol*. 1993;17:1289–1299.

34. Gentile TC, Uner AH, Hutchison RE, et al. CD3+ CD56+ aggressive variant of large granular lymphocyte leukemia. *Blood*. 1994;84:2315–2321.
35. Hirose Y, Masaki Y, Yoshioka R, et al. Aggressive natural killer cell lymphoproliferative disorder associated with Epstein-Barr viral RNA. *Am J Hematol*. 1997;54:314–320.
36. Ohnuma K, Toyoda Y, Nishihira H, et al. Aggressive natural killer (NK) cell lymphoma: report of a pediatric case and review of the literature. *Leuk Lymphoma*. 1997;25:387–392.
37. Chan JK. Splenic involvement by peripheral T-cell and NK-cell neoplasms. *Semin Diagn Pathol*. 2003;20:105–120.
38. Loughran TP Jr. Clonal diseases of large granular lymphocytes. *Blood*. 1993;82:1–14.
39. Ino T, Tsuzuki M, Okamoto M, et al. Acute leukemia with the phenotype of a natural killer/T-cell bipotential precursor. *Ann Hematol*. 1999;78:43–47.
40. Emile JF, Boulland ML, Haioun C, et al. CD5– CD56+ T-cell receptor silent peripheral T-cell lymphomas are natural killer cell lymphoma. *Blood*. 1996;87:1466–1473.
41. Kanavaros P, Boulland ML, Petit B, et al. Expression of cytotoxic proteins in peripheral T-cell and natural killer-cell (NK) lymphomas: association with extranodal site, NK or T_{gd} phenotype, anaplastic morphology and CD30 expression. *Leuk Lymphoma*. 2000;38:317–326.
42. Takeshita M, Yamamoto M, Kikuchi M, et al. Angiodestruction and tissue necrosis of skin-involving CD56+ NK/T-cell lymphoma are influenced by expression of cell adhesion molecules and cytotoxic granule and apoptosis-related proteins. *Am J Clin Pathol*. 2000;113:201–211.
43. Isaacson PG, Chott A, Ott G, et al. Enteropathy-associated T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:289–291.
44. Suzuki R, Suzumiya J, Nakamura S, et al. Aggressive natural kill-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. *Leukemia*. 2004;18:763–770.
45. Mitsui T, Maekawa I, Yamane A, et al. Characteristic expansion of CD45RA+ CD27– CD28– CCR7– lymphocytes with stable natural killer (NK) receptor expression in NK-and T-cell type lymphoproliferative disease of granular lymphocytes. *Br J Haematol*. 2004;126:55–62.
46. Zambello R, Semenzato G. Natural killer receptors in patients with lymphoproliferative diseases of granular lymphocytes. *Semin Hematol*. 2003;40:201–212.
47. Epling-Burnette PK, Painter JS, Chaurasia P, et al. Dysregulated NK receptor expression in patients with lymphoproliferative disease of granular lymphocytes. *Blood*. 2004;103:3431–3439.
48. Mori KL, Egashira M, Oshimi K. Differentiation stage of natural killer cell-lineage lymphoproliferative disorders based on phenotypic analysis. *Br J Haematol*. 2001;115:225–228.
49. Caligiuri MA. Human natural killer cells. *Blood*. 2008;112:461–469.
50. Oshimi K. Lymphoproliferative disorders of natural killer cells. *Int J Hematol*. 1996;63:279–290.
51. Ohsawa M, Nakatsuka SI, Kanno H, et al. Immunophenotypic and genotypic characterization of nasal lymphoma with polymorphic reticulosis morphology. *Int J Cancer*. 1999;81:865–870.
52. Wong KF. Genetic changes in natural killer cell neoplasms. *Leuk Res*. 2002;26:977–978.
53. Kohrt H, Advani R. Extranodal natural killer/T-cell lymphoma: current concepts in biology and treatment. *Leuk Lymphoma*. 2009;50:1773–1784.
54. Wong KF, Chan JKC, Kwong YL. Identification of del(6)(q21q25) as a recurring chromosomal abnormality of putative NK cell lymphoma/leukemia. *Br J Haematol*. 1997;97:922–926.
55. Tien HF, Su IJ, Tang JL, et al. Clonal chromosomal abnormalities as direct evidence for clonality in nasal T/natural killer cell lymphomas. *Br J Haematol*. 1997;97:621–625.
56. Ng CS, Lo STH, Chan JKC. Peripheral T and putative natural killer cell lymphoma commonly coexpress CD95 and CD95 ligand. *Hum Pathol*. 1999;30:48–53.
57. Huang Y, de Reynies A, de Leval L, et al. Gene expression profiling identified emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. *Blood*. 2010;115:1226–1237.
58. Iqbal J, Kucuk C, deLeeuw RJ, et al. Genomic analyses reveal global functional alterations that promote tumor growth and novel tumor suppressor genes in natural killer-cell malignancies. *Leukemia*. 2009;23:1139–1151.
59. Yamanaka Y, Tagawa H, Takahashi N, et al. Aberrant overexpression of microRNAs activate AKT signaling via down-regulation of tumor suppressors in natural killer-cell lymphoma/leukemia. *Blood*. 2009;114:3265–3275.
60. Okuno SH, Tefferi A, Hanson CA, et al. Spectrum of diseases associated with increased proportions or absolute numbers of peripheral blood natural killer cells. *Br J Haematol*. 1996;93:810–812.
61. Sun T, Susin M, Brody J, et al. T-cell lymphoma associated with natural killer-like T-cell reaction. *Am J Hematol*. 1998;57:331–337.

CASE 39

Adult T-Cell Leukemia/Lymphoma

CASE HISTORY

A 47-year-old heterosexual man was admitted to the hospital because of generalized lymphadenopathy for 2 weeks' duration. The patient was in generally good health until 2 weeks before admission, when he started to notice generalized skin rash, lymphadenopathy, and fever. The skin lesion did not respond to diphenhydramine (Benedryl). The patient was born in Colombia and migrated to this country 10 years previously.

Physical examination revealed generalized discrete and confluent erythematous skin rashes, generalized lymphadenopathy, and hepatosplenomegaly. Laboratory data showed a total leukocyte count of 7,700/mL with 51% neutrophils and 37% lymphocytes. His hemoglobin was 15.3 g/dL, and platelets 201,000/mL. The chemistry profile demonstrated generally elevated enzyme levels, including lactate dehydrogenase 363 U/L, alanine aminotransferase 63 U/L, and alkaline

phosphatase 466 U/L, as well as a high serum calcium level (13.5 mg/dL). Viral serologic tests showed positive antibodies for cytomegalovirus, Epstein-Barr virus (IgG), hepatitis B virus, and human T-cell leukemia virus type 1 (HTLV-1).

Chest x-ray examination revealed enlarged hilar lymph nodes, a finding confirmed by computed tomography. The lymph node biopsy was reported as diffuse mixed large- and small-cell lymphoma with positive T-cell markers. The bone marrow aspirate was, however, nondiagnostic. The patient responded well to chemotherapy and was discharged 3 weeks after admission to be followed in the outpatient clinic.

FLOW CYTOMETRY FINDINGS

Peripheral blood: B-cell markers: CD19 1%, CD20 1%, HLA-DR 4%. T-cell markers: CD2 98%, CD3 96%, CD4 93%, CD5 95%, CD7 15%, CD8 5%, CD25 (interleukin 2 receptor [IL-2R]) 8%. Monocyte marker: CD14 7% (Fig. 6.39.1).

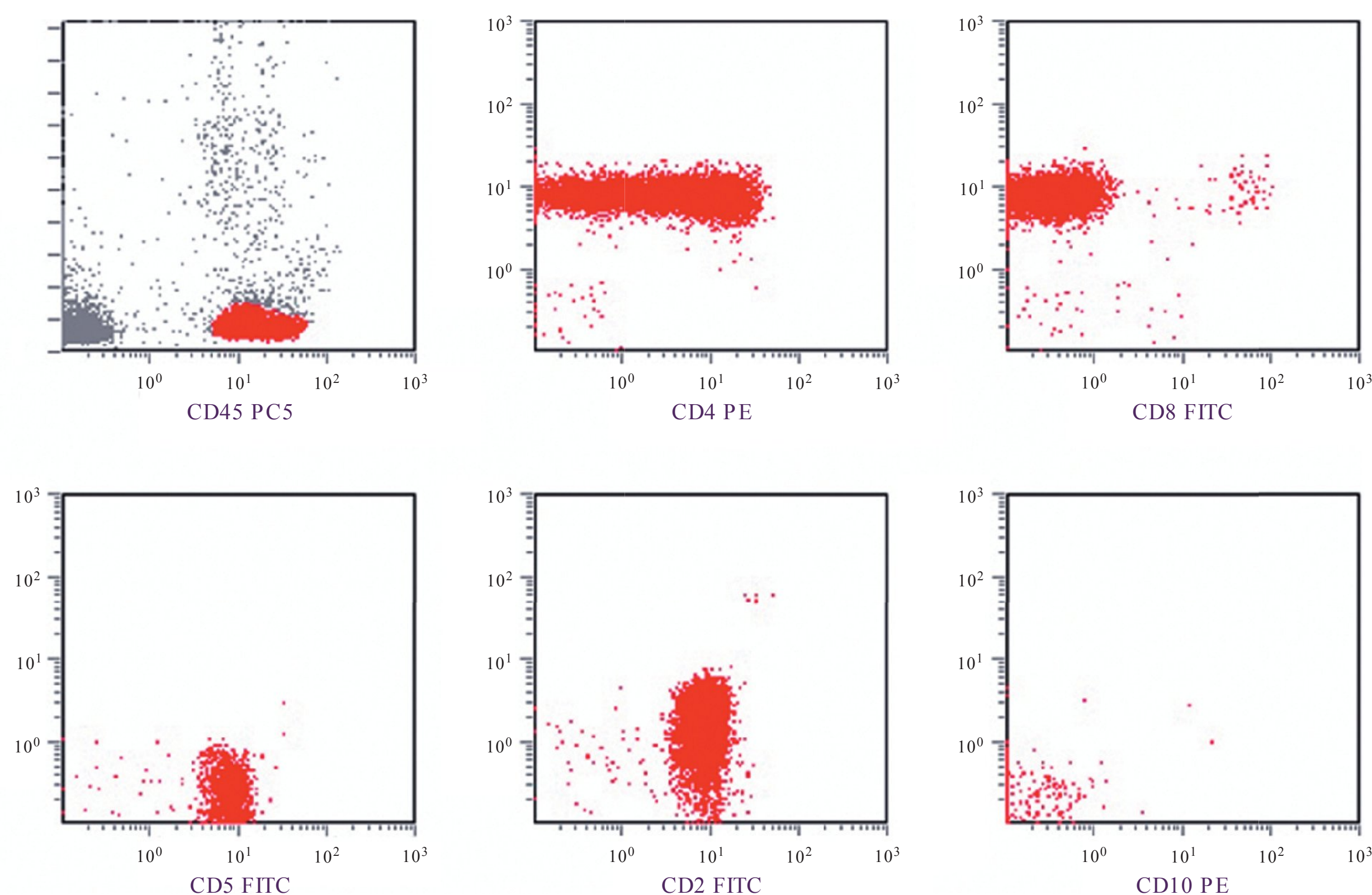


FIGURE 6.39.1 Flow cytometric histograms show positive reactions to CD2, CD3, CD4, CD5, and CD25, but negative reactions to CD7, CD8, CD10, and CD19. This immunophenotype is characteristic of, though not diagnostic for, adult T-cell leukemia/lymphoma. These histograms are not from the current case. SS, side scatter; PC5, phycoerythrin-cyanin 5; ECD, phycoerythrin-Texas Red; PE, phycoerythrin; FITC, fluorescein isothiocyanate.

CYTOCHEMICAL STAINS

Acid phosphatase: Focal paranuclear staining was sensitive to tartrate treatment. Periodic acid-Schiff (PAS): A few lymphocytes showed fine PAS-positive granules.

DISCUSSION

The first cases of adult T-cell leukemia/lymphoma (ATCL) were reported from the Kyushu Islands in Japan in 1977 (1). The circumscribed geographic distribution of the Japanese patients in southwestern Japan raised the question of a viral cause, which was later proved to be a retrovirus, HTLV-1 (2,3). Subsequently, patients with similar symptoms were reported from the Caribbean Basin (4,5) and from the southeastern United States (6,7). Seroepidemiologic studies have indicated that HTLV-1 is also endemic in other Asian regions, such as Taiwan and Okinawa. ATCL cases have subsequently been reported from Hawaii, Britain, and the European continent (8). Current epidemiologic studies have confirmed that the major endemic areas for HTLV-1 infection are the Caribbean, southern Japan, Central and South Africa, and South America. Patients found in North America and Europe are mainly confined to certain immigrant groups and intravenous drug users (9). In the endemic areas, the seroprevalence varies between 0.1% and 30% (9). In Japan, the cumulative incidence of ATCL is estimated to be 4% among HTLV-1 carriers (10). In one study in Japan, ATCL accounted for 48% of all T-cell lymphomas (11).

The median age at diagnosis is about 35 years for patients in the United States, 40 years in the Caribbean Basin, and 60 years in Japan. HTLV-1 can be transmitted through sexual intercourse, blood transfusion, and breast feeding. However, most ATCL patients in endemic areas contract the disease through breast milk, and the disease has a long latent period of several decades after the initial infection (12). Transmission requires transfer of HTLV-1-infected cells and not free virus.

Morphology

The pathognomonic feature of ATCL is the presence of polylobated nuclei in the tumor cells (flower cells) (Fig. 6.39.2) (12,13). The nuclear chromatin is moderately condensed, and nucleoli are inconspicuous. The cytoplasm is slightly to moderately basophilic. Several morphologic variants including pleomorphic small-, medium-, and large-cell types; anaplastic; and a rare form resembling angioimmunoblastic T-cell lymphoma have been reported (14). Blast-like cells with transformed nuclei and dispersed chromatin may also be present in variable proportions (14). The percentage of atypical leukemic cells present in the peripheral blood varies depending on the clinical forms of ATCL. As will be discussed later, there are four clinical forms of ATCL: acute (55%), chronic (20%), smoldering (5%), and lymphomatous (20%) (9).

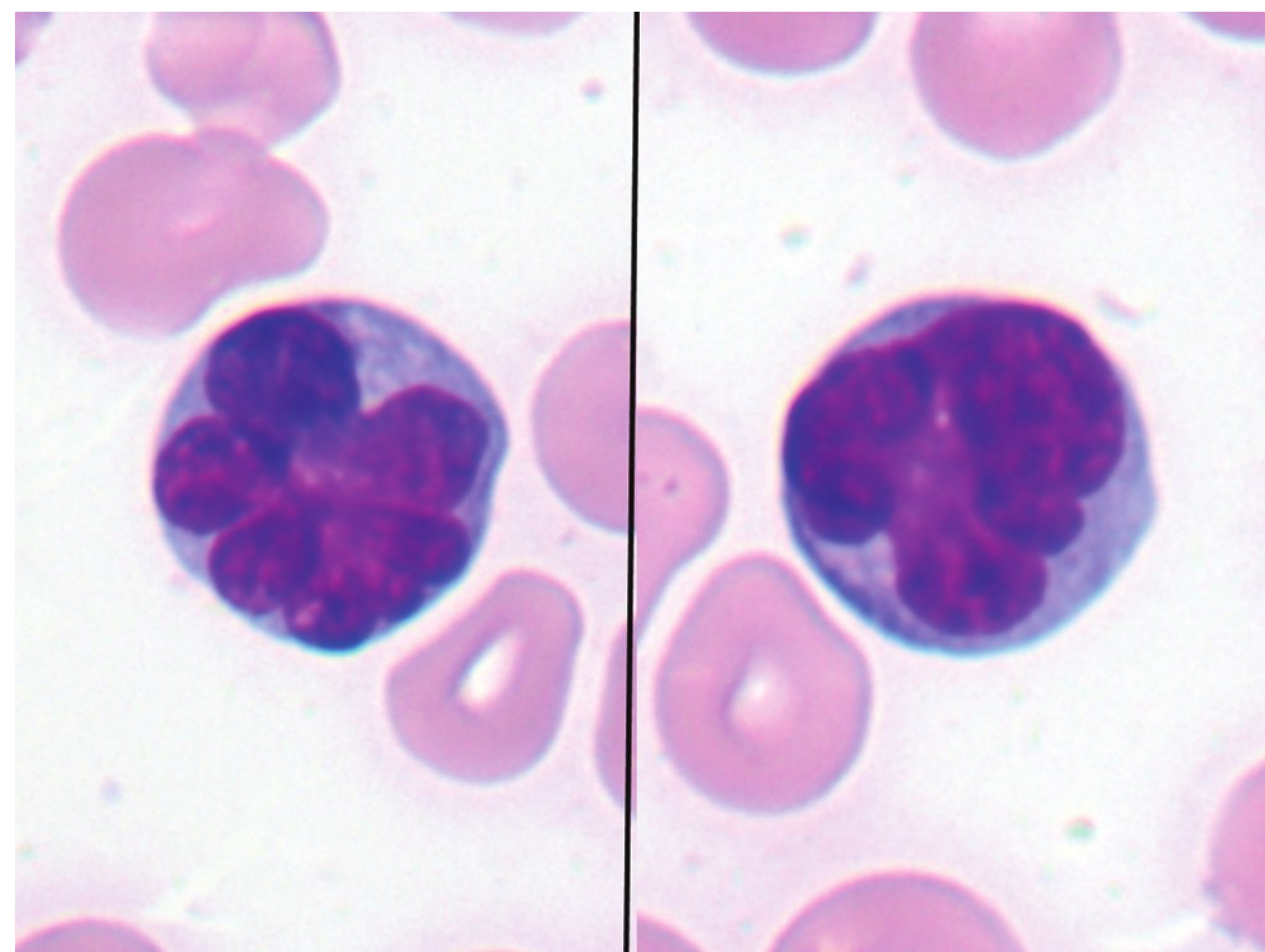


FIGURE 6.39.2 Two ATCL cells with hyperlobated nucleus and basophilic cytoplasm (flower cells) are from the peripheral blood of an ATCL patient. Wright-Giemsa, 300× magnification.

In the acute form, there are numerous atypical lymphoid cells in the blood, and those cells show marked variation in size and in shape (12). In the chronic form, the tumor cells are relatively uniform in cell size and in nuclear configuration. The percentage of atypical cells in the chronic form is lower than in the acute form. In the smoldering form, there are only 0.5% to 3% atypical cells present in the blood. The tumor cells are relatively large with indented, cleft, or bilobed nuclei (12). These tumor cells are sometimes difficult to distinguish from Sézary cells.

The lymphomatous form shows no leukemic cells in the peripheral blood, but small pleomorphic lymphoid cells equivalent to the flower cells may predominate or may be admixed with large transformed cells (14,15). Giant cells with convoluted or cerebriform nuclear contours may also be seen (14,15). Hodgkin-like histology may be detected in the lymph node of some cases (16). This variant shows Epstein-Barr virus positive B-lymphocytes with Reed-Sternberg-like cell morphology and is considered to be secondary to the underlying immunodeficiency seen in ATCL patients (14,17). However, it is associated with a less aggressive clinical course (14). When the lymph nodes are involved, the normal architecture is frequently completely replaced by pleomorphic tumor cells of varying morphology as described above (Fig. 6.39.3) (17).

Skin lesions are a constant feature of ATCL and may mimic mycosis fungoides/Sézary syndrome (MF/SS) by showing plaque, tumor, and erythroderma forms (17). Only the papular form is specific for ATCL. Skin biopsy may show dermal infiltration by small or large tumor cells, including CD30-positive large cells, mimicking Ki-1 anaplastic large-cell lymphoma (Fig. 6.39.4). Pautrier microabscesses are present in >50% of ATCL cases and thus do not constitute an absolute index for distinguishing ATCL from MF/SS (11).

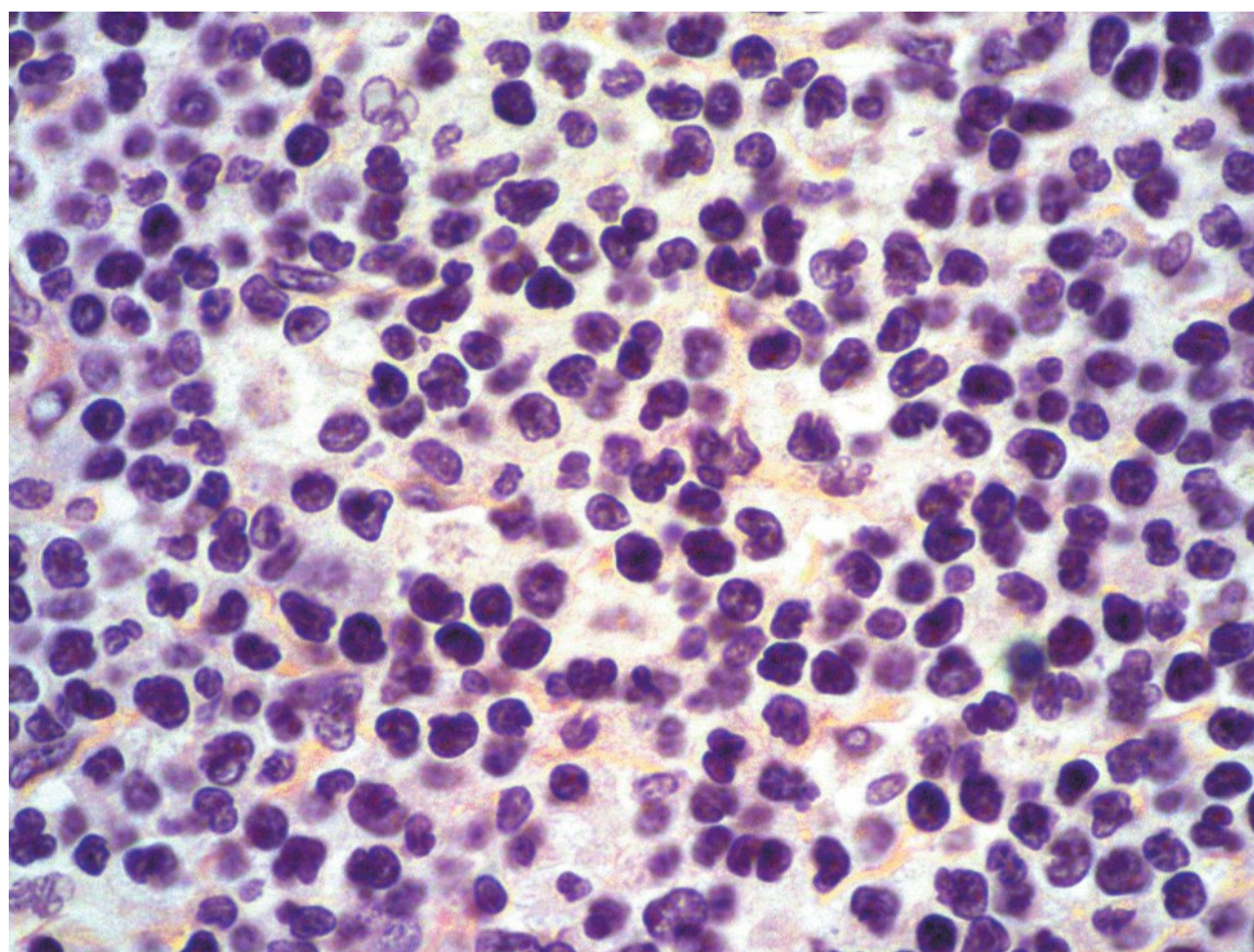


FIGURE 6.39.3 Lymph node biopsy reveals small tumor cells with irregular nuclear configuration, replacing normal architecture. Hematoxylin and eosin, 60× magnification. (Case contributed by Dr. I. J. Su of the National Taiwan University).

Immunophenotype

Immunophenotyping in ATCL is characterized by its helper T-cell phenotype (CD4+, CD8−), consistent presence of T-cell activation marker, CD25 (Tac antigen or IL-2R), and frequent loss of CD7, a pan-T-cell antigen (18,19). Other positive T-cell markers include CD2, CD3, and CD5. The use of CD3/side-scatter gating in flow cytometric study may facilitate the identification of ATCL tumor cells (20).

As a peripheral T-cell neoplasm, terminal deoxynucleotidyl transferase (TdT) and CD1a are consistently negative in ATCL. B-cell markers are always negative, but myeloid markers are expressed in an ATCL cell line (21). An

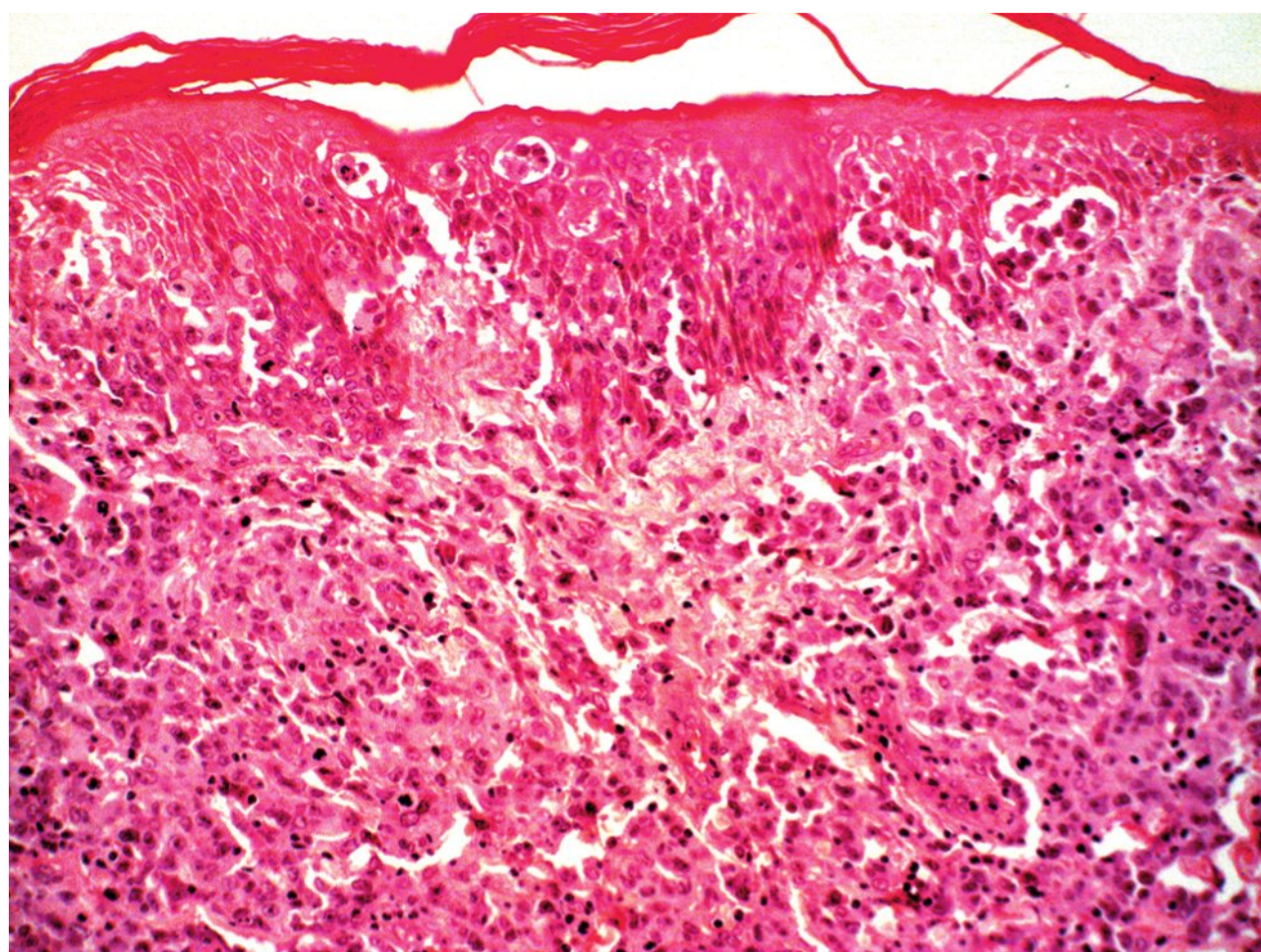


FIGURE 6.39.4 Skin biopsy of an ATCL patient shows multiple Pautrier microabscesses in the epidermis. The dermis is extensively infiltrated by large tumor cells. Hematoxylin and eosin, 20× magnification. (Case contributed by Dr. I. J. Su of the National Taiwan University).

interesting finding related to the helper phenotype is that among the CD4 monoclonal antibodies, ATCL cells react only to Leu 3a and OKT-4A but not to OKT-4, a phenomenon that represents an epitope deficiency on the ATCL cells (22).

ATCL cells are also characterized by their frequent expression of activated cell antigens (23). A constant feature is the expression of CD25, the Tac antigen, which is related to the mechanism of leukemogenesis of T cells (24). In addition, high percentages of positivity with CD28, CD38, CD71, and Ki-67 are frequently demonstrated in ATCL cells in the acute stage and, to a much lesser degree, in the chronic stage (23). On the contrary, the expression of HLA-DR is higher in the chronic than in the acute stage of ATCL. Furthermore, the activated antigens are usually positive in a higher percentage of ATCL cells in the lymph nodes than in the peripheral blood, which is suggestive of a preferential proliferation of ATCL cells in the lymph node (23). The ATCL cells that infiltrate the skin lack CD29 and CD45RA, whereas tumor cells in the peripheral blood and lymph node may be positive for these antigens (17). CD52 is usually positive in the ATCL cells; those cases can thus be treated with anti-CD52 humanized antibody (alemtuzumab or Campath) (15). A transcription factor, FOXP3, has been reported in 68% of ATCL cases, but no other T-cell lymphoma subtypes express this marker (15). The presence of FOXP3, CD25, and CD4 on the tumor cells suggests that ATCL cells are probably of regulatory T (Treg) cell origin.

The immunophenotype of ATCL overlaps with that of MF/SS, including CD25, which can be present in some MF/SS cases (25). The Leu-8 (CD62 ligand [CD62L]) antibody (which is absent on MF/SS cells, but present on ATCL cells) used to be considered the most helpful marker for differentiation (17,26). However, it is seldom used now in clinical laboratories.

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometric analysis may demonstrate a CD4-predominant T-cell phenotype with selective loss of CD7 and positive CD25 staining. Immunohistochemistry is not as convenient as flow cytometry because staining for multiple T-cell markers on the same cells cannot be done and CD25 antibody is not readily available.

Molecular Genetics

In the course of HTLV-1 infection, the viral genome is integrated into the nuclear DNA sequences of the host cells, becoming a provirus that can be replicated along with the host's cellular genome (27). Therefore, although HTLV-1 can be isolated from clinical specimens, it is easier to identify the viral genome in host cellular DNA. Frequently, a monoclonal or an oligoclonal pattern of HTLV-1 integration into cellular DNA can be demonstrated by Southern blotting technique in DNA extracted from ATCL cells. Currently, HTLV-1 can also be detected by the polymerase chain reaction (PCR) or reverse transcriptase PCR technique using a probe specific for the HTLV-1 pol sequences or the pX gene (19,28,29).

HTLV-1 does not contain an oncogene, but its gene product, the pX protein, may act via a lymphokine, probably the ATCL-derived factor, to enhance the expression of IL-2R, which responds to the T-cell growth factor (8,24). Another theory is that the pX region in the HTLV-1 genome encodes two regulatory proteins, Tax and Rex. The Tax protein could activate the transcription of IL-2 and IL-2R α -chain gene in vitro (19,30). Through the above mechanisms, the neoplastic T cells proliferate and the malignant clone expands.

Currently, the Tax protein is widely regarded as the key factor in the tumorigenesis of ATCL, but a unified concept of the detailed mechanism has not yet been established (10,12,31–33). One of the theories is that Tax can inactivate p53, a tumor-suppressor protein that has the function of regulating the cell cycle and apoptosis and maintaining the cellular genome integrity (31). Due to the inactivation of p53, Tax can immortalize the HTLV-1-infected cells and destabilize their genome. Subsequent oncogene involvement or other type of chromosomal aberrations is needed to transform the infected cells into neoplastic cells. This may explain why there is a long latent period between HTLV-1 infection and the development of ATCL. Another theory emphasizes the activation of the nuclear factor- κ B (NF- κ B) by Tax protein (12). The function of NF- κ B includes promotion of cell proliferation, angiogenesis, and resistance to apoptosis. A recent study found overexpression of the CARMA1 gene in lymphoma patients with 7p22 amplification (32). This gene also activates NF- κ B and may be complementary to the same function of Tax. However, the function of Tax is multifactorial, including activation of transcription factors, modification of signal transduction pathways, alteration of tumor suppressor protein functions, modulation of cell-cycle checkpoint proteins, interference with DNA repair, and inhibition of apoptosis (10).

Cytogenetic abnormalities are frequently seen in ATCL patients, but a specific cytogenetic marker(s) is lacking. For instance, 53% of the ATCL prodromal group had chromosomally abnormal clones (34). High frequency of allelic loss is seen on chromosomes 6q (41%) and 17p (48%) in patients with acute and/or lymphomatous ATCL (35). The authors suggested that a novel tumor suppressor gene on chromosome arm 6q and the p53 gene on chromosome arm 17p probably have an important role in the development of acute and/or lymphomatous ATCL (35). Indeed, p53 overexpression is observed in almost 50% of aggressive ATCL cases (36). One study found structural abnormalities occur most frequently in chromosome 6 (15). Another study showed that the most common cytogenetic abnormalities in the acute subtype of ATCL were trisomy 3, trisomy 7, and the absence of the X chromosome (8). Chromosomal abnormality is seldom seen in chronic or smoldering ATCL, suggestive of clonal evolution during disease progression.

Recently, the CDKN2 gene on chromosome 9q21 has been considered a tumor suppressor gene, playing an important role in the malignant transformation process induced by HTLV (34). Although alterations in the CDKN2

gene were detected in only 15% to 20% of ATCL patients, most patients with this alteration had a clinically aggressive form. Another study showed that methylation of the CDKN2A gene was more frequently demonstrated in fresh tumor cells isolated from patients with the clinically aggressive form than from those with a less aggressive clinical course (37). Thus, methylation of CDKN2A was found in 47% of patients with the acute form, 73% of patients with the lymphomatous form, 17% of patients with the chronic form, and 17% of patients with the smoldering form. Recently, BCL11B overexpression has also been found in the acute form of ATCL cases (32). All these cytogenetic aberrations may represent the cumulative events after genome destabilization induced by the Tax protein, finally leading to the development of ATCL.

Gene expression profiling (GEP) study has identified overexpression of PIRC5 (survivin), a gene that blocks apoptosis and leads to the resistance of ATCL cells to chemotherapy (15). One GEP study identifies specific gene expression signatures that are associated with the responsiveness of the tumor cells to the AZT/INF α therapy (38). Specifically, this therapy induced prominent upregulation of interferon response genes in vivo, while cell cycle-associated genes are silenced (38). Activating mutation of Notch1 has been found in more than 30% of ATCL patients and this gene may serve as a target for therapeutic intervention (39).

Clinical Manifestations

The characteristic clinical features in ATCL are peripheral lymphocytosis with atypical lymphocytes, skin lesions, lymphadenopathy, and hepatosplenomegaly. Although these features may be seen in other T-cell neoplasms, a positive HTLV-1 serologic test and hypercalcemia are usually more supportive of the diagnosis of ATCL. The international collaborative study on the diagnostic criteria of ATCL was proposed in 1994 (Table 6.39.1) (17). Based on this system, seven diagnostic points indicate a definitive diagnosis, five or six is probable, three or four is possible, and fewer than three points is inconsistent with ATCL (17).

TABLE 6.39.1

Diagnostic Point System for ATCL

Criteria	Points
Hypercalcemia	1
Lymphomatous skin lesion	1
Leukemic phase ($>2\%$ abnormal lymphocytes)	1
T-cell lymphoma or leukemia	2
HTLV-1 antibody	2
CD25-positive tumor cells	1
HTLV-1-positive tumors	2

ATCL, adult T-cell leukemia/lymphoma; HTLV-1, human T-cell leukemia virus type 1; CD, cluster of differentiation.



TABLE 6.39.2

Clinical and Laboratory Findings in Various Clinical Subtypes of ATCL

	Smoldering	Chronic	Lymphomatous	Acute
Anti-HTLV-1 antibody	+	+	+	+
Peripheral lymphocytosis ($\times 10^6/\text{mL}$)	<4	≥ 4	<4	≥ 4
Abnormal T lymphocytes (%)	≥ 5	≥ 5	≤ 1	≥ 5
Polylobated lymphocytes	Rare	Rare	No	Frequent
Bone marrow infiltration	–	+	–	+
Lymphadenopathy	–	+	+	+
Skin lesions	+	+	+	+
Hepatomegaly	–	+	$\pm$	+
Splenomegaly	–	+	$\pm$	+
Lytic bone lesions	–	–	–	+
Pulmonary lesions	–	–	$\pm$	+
Central nervous system lesions	–	–	$\pm$	$\pm$
Gastrointestinal tract lesions	–	–	$\pm$	$\pm$
Hypercalcemia	–	–	–	+
Elevated lactate dehydroge- nase		+	+	+
Elevated alkaline phosphatase	–	–	–	+
Hypoproteinemia	–	–	$\pm$	$\pm$
Hyperbilirubinemia	–	–	–	$\pm$
Anemia	–	$\pm$	$\pm$	–
Thrombocytopenia	–	–	–	$\pm$
Eosinophilia	–	$\pm$	–	$\pm$

ATCL, adult T-cell leukemia/lymphoma; HTLV-1, human T-cell leukemia virus type 1.

The clinical course of ATCL may follow a temporally related spectrum, including preleukemic, smoldering, chronic, and subacute-to-acute stages (8). In the preleukemic stage, patients are asymptomatic with no organ involvement except for the bone marrow, which may or may not be infiltrated by tumor cells. Diagnosis is usually made by incidental findings of lymphocytosis with atypical lymphocytes. These patients are, however, seropositive for HTLV-1, and Southern hybridization or PCR frequently shows monoclonal integration of HTLV-1 provirus into tumor cell DNA. About half of the preleukemic patients recover spontaneously without further progression, whereas the remaining half progress through smoldering, chronic, and subacute-to-acute stage.

In one study, the median proviral DNA level was 212 copies/ 10^5 lymphocytes in the first 3 months after HTLV-1 infection (Time 1), 99 copies in 7 to 14 months after infection (Time 2), and 27 copies after 14 months' infection (Time 3) (40). The HTLV-1 antibody titers were low at Time

1, significantly increased by Time 2, and stable by Time 3. Some ATCL patients may have multiple HTVL integrations. When one tumor cell clone carried multiple copies, these patients had an extremely aggressive clinical course with the infiltration of unusual organs. When only one copy of the provirus was carried by each of the multiple clones, patients showed an indolent clinical course with skin lesions (41).

As mentioned before, ATCL can be divided into four clinical subtypes: Smoldering, chronic, acute, and lymphomatous forms, which may also represent different developmental stages of ATCL (Table 6.39.2) (42). The criteria include percentage of abnormal lymphocytes in the peripheral blood; presence or absence of hypercalcemia; levels of lactate dehydrogenase; and tumor involvement of the lymph nodes, liver, spleen, central nervous system, bone, gastrointestinal tract, skin, and lung. A study from Taiwan also included anemia, thrombocytopenia, eosinophilia, hypoproteinemia, hyperbilirubinemia, and elevated

alkaline phosphatase levels in the differential criteria (18). The serum IL-2R levels can also help to distinguish the clinical subtypes. One study showed that the mean IL-2R level was 9740 U/mL in the acute and lymphomatous subtypes, 1961 U/mL in the chronic subtype, and 788 U/dL in the smoldering subtype (43). In a study of 124 cases of ATCL, the median survival time was 4 months in the acute subtype, 7 months in the lymphomatous subtype, 14 months in the chronic subtype, and 16 months in the smoldering subtype (44).

It should be emphasized that the characteristic hypercalcemia is seen in the acute stage secondary to osteolytic lesions (45). These patients may have abnormal bone scintigraphy, elevated alkaline phosphatase levels, and increased osteoclastic activity in bone marrow biopsy, but the parathyroid hormone, cyclic adenosine monophosphate, prostaglandin, and vitamin D levels in serum are normal or low (7,46). Therefore, the release of an osteoclast-activating factorlike substance by the tumor cells is suspected to be the mechanism of hypercalcemia (46). One study demonstrated a parathyroid hormone-related protein in cellular and extracellular sites of neoplastic tissues by immunohistochemical techniques in six of seven ATCL patients with hypercalcemia and suggested that this substance may be responsible for hypercalcemia (47).

Patients with ATCL are immunosuppressed, which may or may not be related to the heightened suppressor function. As a result, opportunistic infections, such as *Pneumocystis carinii* pneumonia, cytomegalovirus pneumonia, *Candida* sepsis, and various bacterial infections, are common and are frequently the cause of death in ATCL patients (7,8,18). More recently, strongyloidiasis has been found to be associated with HTLV-1 infection (36,48). For some unknown reason, patients with a strongyloides hyperinfection showed a high response rate to chemotherapy and prolonged survival (36). Patients with ATCL are also associated with high incidence of chronic hepatitis B or C (49).

HTLV-1 infection may cause different clinical manifestations: Besides ATCL, there are HTLV-1-associated myelopathy/tropical spastic paresis (HAM/TSP), uveitis, arthropathy, and infectious dermatitis (50). The mechanism for the induction of different clinical entities by HTLV-1 is still unclear. However, it may be related to the defect of the provirus and the dose of infection. Retention of the structural genes by defective proviruses might be a risk factor for HAM/TSP development; retention of the regulatory genes in the pX region by the defective proviruses could be a risk factor for ATCL development (51). Another study showed that patients with ATCL had a higher percentage of HTLV-1-positive cells in the peripheral blood than did patients with HAM/TSP (8% to 93% vs. 3.1% to 8.5%) (52).

The current patient had clinical manifestation of generalized lymphadenopathy, skin rash, laboratory findings of the characteristic lymphoid cells with polyllobated nuclei (flower cells) in the peripheral blood, and hypercalcemia; thus, ATCL should be suspected. Immunophenotyping of the peripheral mononuclear cells showed a predominant helper T-cell phenotype with

TABLE 6.39.3

Salient Features for Laboratory Diagnosis of ATCL

1. Positive HTLV-1 antibodies
2. Monoclonal helper-T-cell phenotype
3. Positive for CD2, CD3, CD4, CD5, and CD25
4. Negative for TdT, CD7, CD8, and CD1
5. Identification of HTLV-1 proviral genome in tumor cell DNA by Southern blotting or PCR
6. Isolation of HTLV-1 virus
7. Hypercalcemia

ATCL, adult T-cell leukemia/lymphoma; HTLV-1, human T-cell leukemia virus type 1; TdT, terminal deoxynucleotidyl transferase; PCR, polymerase chain reaction.

selective loss of CD7, which can be seen in both ATCL and MF/SS. In fact, the morphology of the atypical lymphoid cells in ATCL and SS is similar, and yet the low percentage of CD25-positive cells is more frequently seen in MF/SS. (The percentage of CD25-positive cells in the current case increased gradually to a high level after admission.) However, a positive serology for HTLV-1 and the high serum calcium level are strongly in favor of HTLV-1, even though these features can be seen occasionally in MF/SS. For differential diagnosis of post-thymic T-cell leukemias, the reader is referred to Table 6.20.1 in Case 20. The salient features for laboratory diagnosis of ATCL are summarized in Table 6.39.3.

REFERENCES

1. Uchiyama T, Yodoi J, Sagawa K, et al. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood*. 1977;150:481–492.
2. Yoshida M, Miyoshi I, Hinuma Y. Isolation and characterization of retrovirus from cell lines of human T-cell leukemia and its implication in the disease. *Proc Natl Acad Sci U S A*. 1981;78:6476–6480.
3. Blattner WA, Kalyanaraman VS, Robert-Guroff M, et al. The human type C retrovirus, HTLV, in blacks from the Caribbean, and the relationship to adult T-cell leukemia/lymphoma. *Int J Cancer*. 1982;30:257–264.
4. Catovsky D, Greaves MF, Rose M, et al. Adult T-cell lymphoma-leukemia in blacks from the West Indies. *Lancet*. 1982;1:639–643.
5. Swerdlow SH, Habershaw JA, Rohatiner AZS, et al. Caribbean T-cell lymphoma/leukemia. *Cancer*. 1984;54:687–696.
6. Balyney DW, Jaffe ES, Blattner WA, et al. The human T-cell leukemia/lymphoma. *Blood*. 1983;62:401–405.
7. Bunn PA, Schechter GP, Jaffe E, et al. Clinical course of retrovirus-associated adult T-cell lymphoma in the United States. *N Engl J Med*. 1983;309:257–264.
8. Wachsman W, Golde DW, Chen ISY. HTLV and human leukemia: perspectives 1986. *Semin Hematol*. 1986;23:245–256.
9. Bangham CRM. HTLV-1 infections. *J Clin Pathol*. 2000;53: 581–586.

10. Shuh M, Beilke M. The human T-cell leukemia virus type 1 (HTLV-1): new insights into the clinical aspects and molecular pathogenesis of adult T-cell leukemia/lymphoma (ATLL) and tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM). *Microsc Res Tech*. 2005;68:176–196.
11. Oshima K, Suzumiya J, Kikuchi M. The World Health Organization classification of malignant lymphoma: incidence and clinical prognosis in HTLV-1-endemic area of Fukuoka. *Pathol Int*. 2002;52:1–12.
12. Brunning RD, McKenna RW. *Tumor of the Bone Marrow*. Washington, DC: Armed Forces Institute of Pathology; 1994:301–308.
13. Jaffe ES. Post-thymic T-cell lymphomas. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. 2nd ed. Philadelphia, PA: WB Saunders; 1995:344–389.
14. Ohshima K, Jaffe ES, Kikuchi M. Adult T-cell leukaemia/lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:281–284.
15. Jaffe ES. Adult T-cell leukemia/lymphoma. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:521–531.
16. Ohshima K, Suzumiya J, Kato A, et al. Clonal HTLV-1-infected CD4+ T-lymphocytes and non-clonal non-HTLV-1-associated giant cells in incipient ATLL with Hodgkin-like histologic features. *Int J Cancer*. 1997;72:592–598.
17. Watanabe S. Adult T-cell leukemia/lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1603–1616.
18. Shih LY, Kuo TT, Dunn P, et al. Human T-cell lymphotropic virus type 1 associated adult T-cell leukemia/lymphoma in Taiwan Chinese. *Br J Haematol*. 1991;79:156–161.
19. Takatsuki K. Kenneth MacGredie Memorial Lectureship. Adult T-cell leukemia/lymphoma. *Leukemia*. 1997;11(suppl3):54–56.
20. Yokote T, Akioka T, Oka S, et al. Flow cytometric immunophenotyping of adult T-cell leukemia/lymphoma using CD3 gating. *Am J Clin Pathol*. 2005;124:199–204.
21. Koijumi S, Iwanaga M, Imai S, et al. Expression of myeloid cell phenotypes by a novel adult T-cell leukemia/lymphoma cell line. *J Natl Cancer Inst*. 1992;84:690–693.
22. Uozumi K, Ohno N, Ishizuka K, et al. Adult T-cell leukemia in patients with OKT4 epitope deficiency. *Br J Haematol*. 1991;79:651–652.
23. Shirono K, Haltori T, Hata H, et al. Profiles of expression of activated cell antigens on peripheral blood and lymph node cells from different clinical stages of adult T-cell leukemia. *Blood*. 1989;73:1664–1671.
24. Yodoi J, Uchiyama T. IL-2 receptor dysfunction and adult T-cell leukemia. *Immunol Rev*. 1986;92:135–156.
25. Diamandidou E, Cohen PR, Kurzorck R. Mycosis fungoides and Sézary syndrome. *Blood*. 1996;88:2385–2409.
26. Wood GS. Benign and malignant cutaneous lymphoproliferative disorders including mycosis fungoides. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1183–1233.
27. Gotoh YI, Sugamura K, Hinuma Y. Health carriers of a human retrovirus, adult T-cell leukemia virus (ATLV): demonstration by clonal culture of HTLV-carrying T-cell from peripheral blood. *Proc Natl Acad Sci U S A*. 1982;79:4780–4782.
28. Gessain A, Gaumes E, Feyeux C, et al. The cutaneous form of adult T-cell leukemia/lymphoma in a woman from the Ivory Coast. *Cancer*. 1992;69:1362–1367.
29. Lee SN, Nam E, Cha JH, et al. Adult T-cell leukemia/lymphoma with features of CD30-positive anaplastic large cell lymphoma—a case report. *J Korean Med Sci*. 1997;12:364–368.
30. Lyons SF, Leibowitz DN. The role of human viruses in the pathogenesis of lymphoma. *Semin Oncol*. 1998;25:461–475.
31. Tabakin-Dix Y, Azran I, Schavinky-Khrapunsky Y, et al. Functional inactivation of p53 by human T-cell leukemia virus type 1 Tax protein: mechanisms and clinical implications. *Carcinogenesis*. 2006;27:673–681.
32. Oshiro A, Tagawa H, Ohshima K, et al. Identification of subtype-specific genomic alterations in aggressive adult T-cell leukemia/lymphoma. *Blood*. 2006;107:4500–4507.
33. Nicot C. Current views in HTLV-1-associated adult T-cell leukemia/lymphoma. *Am J Hematol*. 2005;78:232–239.
34. Fujimoto T, Hata T, Itoyama T, et al. High rate of chromosomal abnormalities in HTLV-1 infected T-cell colonies derived from prodromal phase of adult T-cell leukemia: a study of IL-2-stimulated colony formation in methylcellulose. *Cancer Genet Cytogenet*. 1999;109:1–13.
35. Hatta Y, Yamada Y, Tomonaga M, et al. Allelotype analysis of adult T-cell leukemia. *Blood*. 1998;92:2113–2117.
36. Agape P, Copin MC, Cavois M, et al. Implication of HTLV-1 infection, strongyloidiasis, and p53 overexpression in the development, response to treatment, and evolution of non-Hodgkin's lymphoma in an endemic area (Martinique, French West Indies). *J Acquir Immune Defic Syndr Hum Retroviral*. 1999;20:394–402.
37. Nosaka K, Maeda M, Tamiya S, et al. Increasing methylation of the CDKN2A gene is associated with the progression of adult T-cell leukemia. *Cancer Res*. 2000;60:27–35.
38. Alizadeh AA, Bohen SP, Lossos C, et al. Expression profiles of adult T-cell leukemia-lymphoma and associations with clinical responses to zidovudine and interferon alpha. *Leuk Lymphoma*. 2010;51:1157–1158.
39. Pancewicz J, Taylor JM, Datta A, et al. Notch signaling contributes to proliferation and tumor formation of human T-cell leukemia virus type 1-associated adult T-cell leukemia. *Proc Natl Acad Sci USA*. 2010;107:16619–16624.
40. Manns A, Miley WJ, Wilks RJ, et al. Quantitative proviral DNA and antibody levels in the natural history of HTLV-1 infection. *J Infect Dis*. 1999;180:1487–1493.
41. Shimamoto Y. Clinical indications of multiple integrations of human T-cell lymphotropic virus type I proviral DNA in adult T-cell leukemia/lymphoma. *Leuk Lymphoma*. 1997;27:43–51.
42. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukemia-lymphoma: a report from the lymphoma study group (1984–87). *Br J Haematol*. 1991;79:428–437.
43. Araki K, Harada K, Nakamoto K, et al. Clinical significance of serum soluble IL-2R levels in patients with adult T-cell leukemia (ATL) and HTLV-I carriers. *Clin Exp Immunol*. 2000;119:259–263.
44. Setoyam M, Katahira Y, Kanzaki T. Clinicopathologic analysis of 24 cases of adult T-cell leukemia/lymphoma with cutaneous manifestations: the smoldering type with skin manifestations has a poorer prognosis than previously thought. *J Dermatol*. 1999;26:785–790.
45. Uchida T, Kinoshita T, Murate T, et al. CDKN2 (MTS1/p16INK4A) gene alterations in adult T-cell leukemia/lymphoma. *Leuk Lymphoma*. 1998;29:27–35.
46. Kiyokawa T, Yamaguchi K, Takaya M, et al. Hypercalcemia and osteoclast proliferation in adult T-cell leukemia. *Cancer*. 1987;59:1187–1191.

47. Moseley JM, Danks JA, Grell V, et al. Immunocytochemical demonstration of PTHrP protein in neoplastic tissue of HTLV-I positive human adult T-cell leukemia/lymphoma: implications for the mechanism of hypercalcemia. *Br J Cancer*. 1991;64:745–748.
48. Marsh BJ. Infectious complications of human T-cell leukemia/ lymphoma virus type I infection. *Clin Infect Dis*. 1996;23:139–145.
49. Lee CW, Chang MC, Chang YF, et al. Adult T-cell leukemia/ lymphoma in Taiwan: an analysis of 17 patients and review of the literature. *Asia Pac J Clin Oncol*. 2010;6:161–164.
50. Uchiyama T. Human T-cell leukemia virus type I (HTLV-I) and human diseases. *Annu Rev Immunol*. 1997;15:15–37.
51. Renjifo B, Chou K, Soto Ramirez L, et al. Human T-cell leukemia virus type I (HTLV-I) molecular genotypes and disease outcome. *J Acquir Immune Defic Syndr Hum Retroviral*. 1996;13(suppl 1):S146–S153.
52. Hashimoto K, Higuchi I, Osame M, et al. Quantitative in situ PCR assay of HTLV-I infected cells in peripheral blood lymphocytes of patients with ATL, HAM/TSP and asymptomatic carriers. *J Neurol Sci*. 1998;159:67–72.

CASE 40

Hepatosplenic T-Cell Lymphoma

CASE HISTORY

A 28-year-old man was admitted to the hospital because of fever of unknown origin. The patient had had a low-grade fever for 10 days without other symptoms. On admission, he appeared chronically ill with a temperature of 38.4°C. No lymph node was palpable. Complete blood cell count showed a hemoglobin level of 8.1 g/dL, hematocrit 24.4%, and leukocyte count 15,800/mL with 72% segmented neutrophils, 6% bands, 22% lymphocytes, and 1% nucleated erythrocytes. His platelet count was 127,000/mL and reticulocytes 8.5%. Blood chemistry tests on admission showed an alkaline phosphatase level of 152 U/L, lactate dehydrogenase level of 540 U/L, and alanine amino transferase level of 58 U/L. Results of chest x-ray were within normal limits. Cultures for bacteria and fungi were negative. The result of a skin tuberculin test was negative.

Computed tomography of the abdomen revealed hepatosplenomegaly. Barium enema and upper gastrointestinal series were normal. A bone marrow biopsy showed a hypercellular bone marrow with erythroid hyperplasia and focal infiltration by immature lymphoid cells. Hemoglobin electrophoresis was consistent with diagnosis of thalassemia. Because of persistent fever, bone marrow abnormality, and splenomegaly, the patient underwent splenectomy, and a liver biopsy was performed. A lymph node biopsy was subsequently taken. After the operation, the patient started on chemotherapy, which resulted in normalization of his temperature and liver function. He subsequently had several episodes of recurrent fever, and leukopenia necessitated repeated chemotherapy. The patient was finally referred for allogeneic bone marrow transplantation.

FLOW CYTOMETRY FINDINGS

Splenectomy specimen: B-cell markers: IgG 14%, IgA 9%, IgM 11%, k 12%, l 10%, CD19 11%, CD20 10%, HLA-DR 19%.

T-cell markers: CD3 79%, CD5 12%, CD7 4%, CD4 0%, CD8 0%. Monocyte marker: CD14 11%.

Liver biopsy: B-cell markers: k 5%, l 5%, CD19 0%, CD20 0%, HLA-DR 8%. T-cell markers: CD3 80%, CD5 5%, CD7 8%. Monocyte marker: CD11c 16%.

CYTOCHEMISTRY

Tumor cells in the liver biopsy were negative for terminal deoxynucleotidyl transferase.

IMMUNOGENOTYPING

No rearrangement was demonstrated in the Ig heavy-chain gene, and k and l light-chain genes. T-cell receptor (TCR) b-chain gene rearrangement was also negative. TCRg-chain gene rearrangement analysis was not performed.

DISCUSSION

On the basis of gene arrangement, T cells can be divided into $\alpha\beta$ T cells and $\gamma\delta$ T cells. In the peripheral blood, only 1% to 5% of lymphocytes are $\gamma\delta$ T cells (1). $\gamma\delta$ T cells may express a Cg1-containing TCR or Cg2-containing TCR. These two receptors can be roughly distinguished by the anti-Vd1 (A13) and anti-Vd2 (BB3) antibodies, respectively (2). The Vd1 subset is preferentially distributed in the spleen and thymus, whereas the Vd2 subset is preferentially distributed in the peripheral blood, lymph nodes, tonsils, skin, and mucosa (3,4). The highest concentration of $\gamma\delta$ T cells is located in the spleen (2). The $\gamma\delta$ TCR seems to direct T cells homing to the splenic sinusoids (5,6), which is a characteristic histologic feature of hepatosplenic $\gamma\delta$ T-cell lymphoma, as defined by the Revised European American classification of Lymphoid neoplasms. This is a rare disease; <100 cases have been reported (3,7–10).

Several cases of hepatosplenic ab T-cell lymphoma have also been reported recently, and they are indistinguishable morphologically and clinically from the hepatosplenic gd T-cell lymphoma (11–14). Therefore, the World Health Organization (WHO) classifies these two tumors into a single entity, the hepatosplenic T-cell lymphoma (HSTCL), and cases with the ab T-cell phenotype is considered a variant (15,16).

gd T cells are similar to natural killer (NK) cells in several respects. They may assume the morphology of large granular lymphocytes (2), and express both the NK markers (CD56 and CD16) and the cytotoxic proteins, including T-cell intracellular antigen-1 (TIA-1), granzyme B, and perforin. However, gd T cells can be distinguished from NK cells by the presence of TCR gene rearrangement and by the expression of CD3 and the TCR proteins.

As TCRgd-chain genes arrange before TCRAb-chain genes do, the gd T-cell neoplasms are mostly seen in precursor T-cell lymphoma/leukemia; only a small percentage of peripheral T-cell lymphomas are of gd origin (1). Among the peripheral gd T-cell lymphomas, hepatosplenic gd T-cell lymphoma is the prototype, but rare cases can be detected in the nasal, respiratory, gastrointestinal, and cutaneous sites (2). The primary cutaneous gd T-cell lymphoma is categorized as a separate disease entity in the 2008 WHO classification (17).

Morphology

Histologically, HSTCL is characterized by the presence of sinusoidal infiltration of the tumor cells in the spleen, liver, and bone marrow without involvement of the lymph nodes. The most characteristic features are usually demonstrated in the spleen. The spleen is often markedly enlarged, frequently in the range between 1,000 and 3,500 g (1). The cut surface is characteristically a homogeneous purple-red color, reflecting the extensive red pulp infiltration. No nodular pattern is present because the white pulp is usually atrophic without tumor cell infiltration. Microscopically, there is extensive tumor cell infiltration in the red pulp cords of Billroth and the sinuses (Fig. 6.40.1). The red pulp sinuses are dilated,

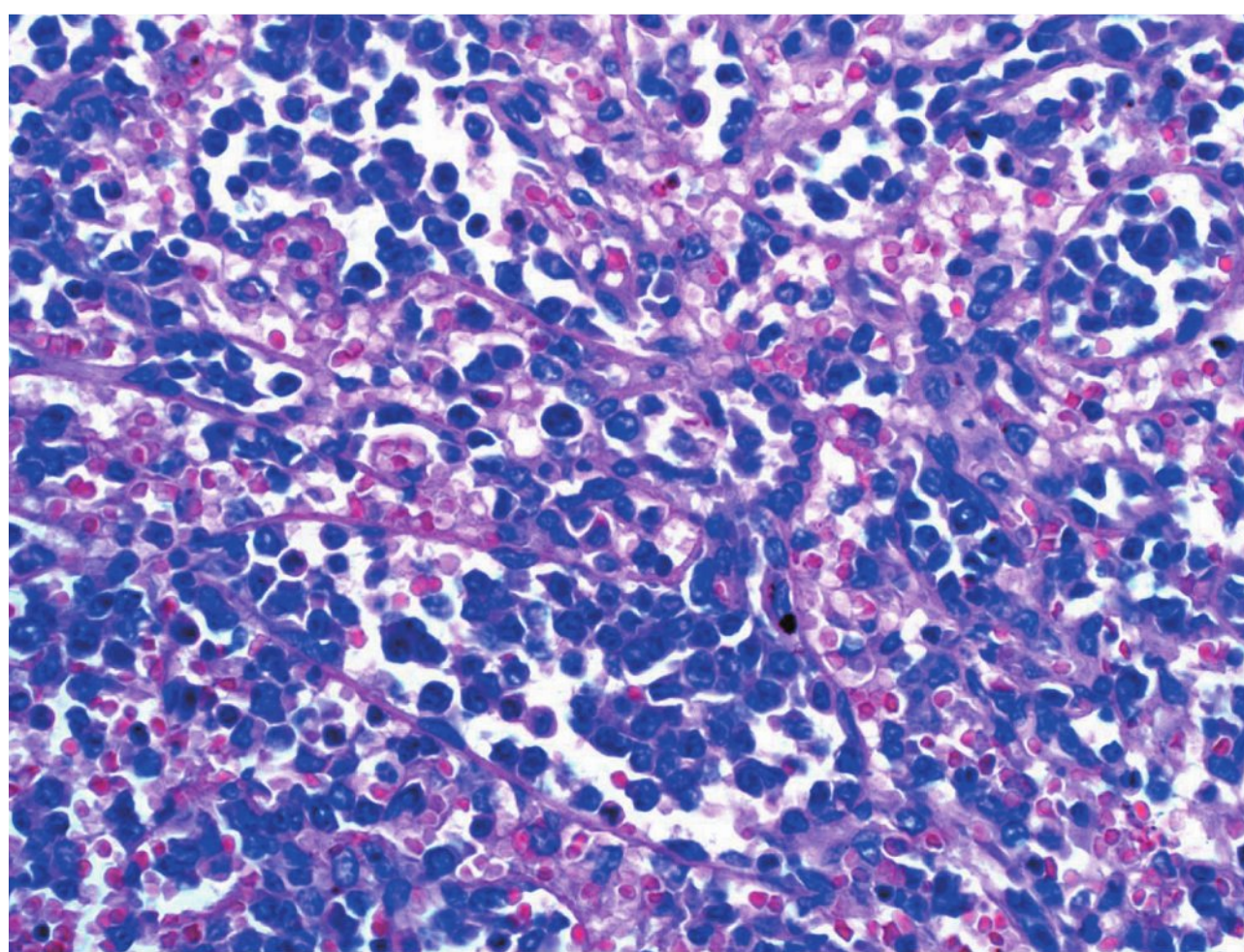


FIGURE 6.40.1 Splenectomy specimen shows dilated sinuses filled with lymphoma cells. Hematoxylin and eosin, 40× magnification.

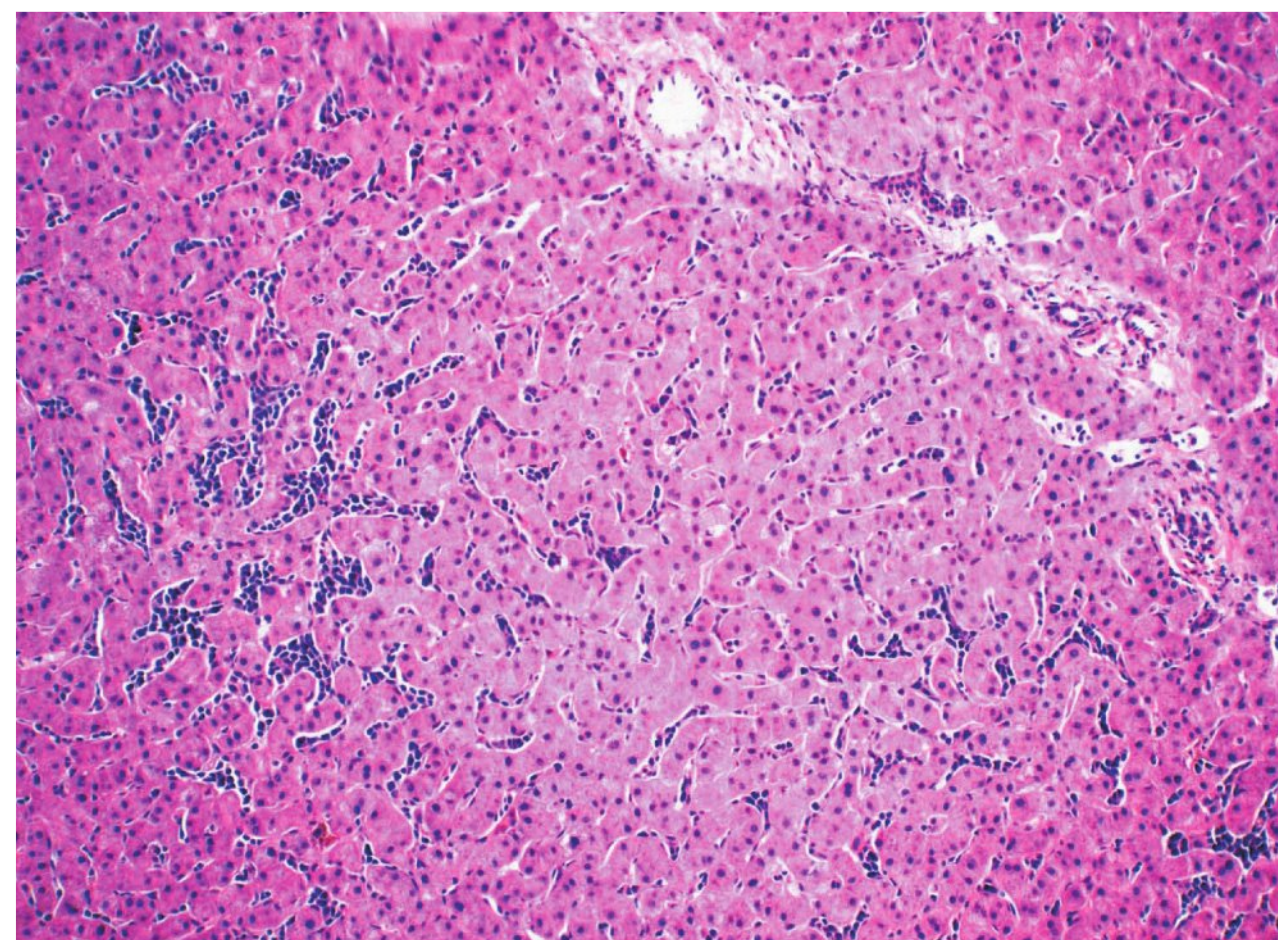


FIGURE 6.40.2 Liver biopsy shows sinusoidal infiltration by lymphoma cells, whereas the portal areas are devoid of tumor cell infiltration. Hematoxylin and eosin, 10× magnification.

containing clusters of monomorphic tumor cells; this feature is characteristic though not diagnostic of this disease.

The tumor cells are of small-to-medium size, with a moderate amount of cytoplasm. Although normal gd T cells contain azurophilic cytoplasmic granules, these granules are seen only in a few cases of HSTCL. The nuclei can be irregular with slightly dispersed chromatin and inconspicuous nucleoli (1,3). When the disease advances, large tumor cells or blast forms may appear in the bone marrow (1,2). Histiocytosis, which may be accompanied by hemophagocytosis, is seen in the spleen in a small number of cases. Hepatomegaly without nodules is also a constant feature in this disease. Similar to the splenic lesion, sinusoidal infiltration by tumor cells is characteristic (Figs. 6.40.2 and 6.40.3). The portal areas are usually spared with tumor cells, or they show mild infiltration.

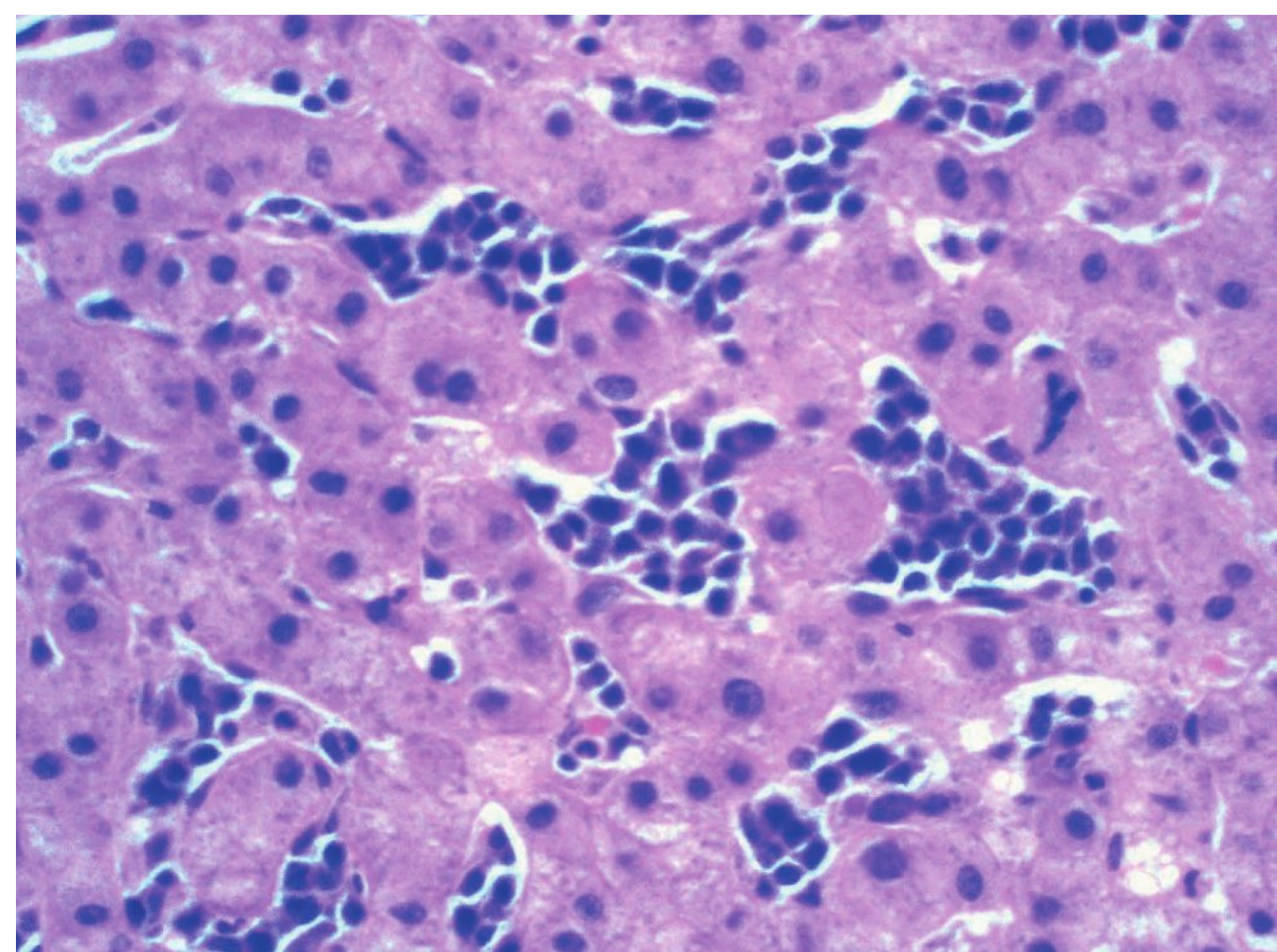


FIGURE 6.40.3 Higher magnification of the liver biopsy shows clusters of tumor cells in the sinuses. Hematoxylin and eosin, 40× magnification.

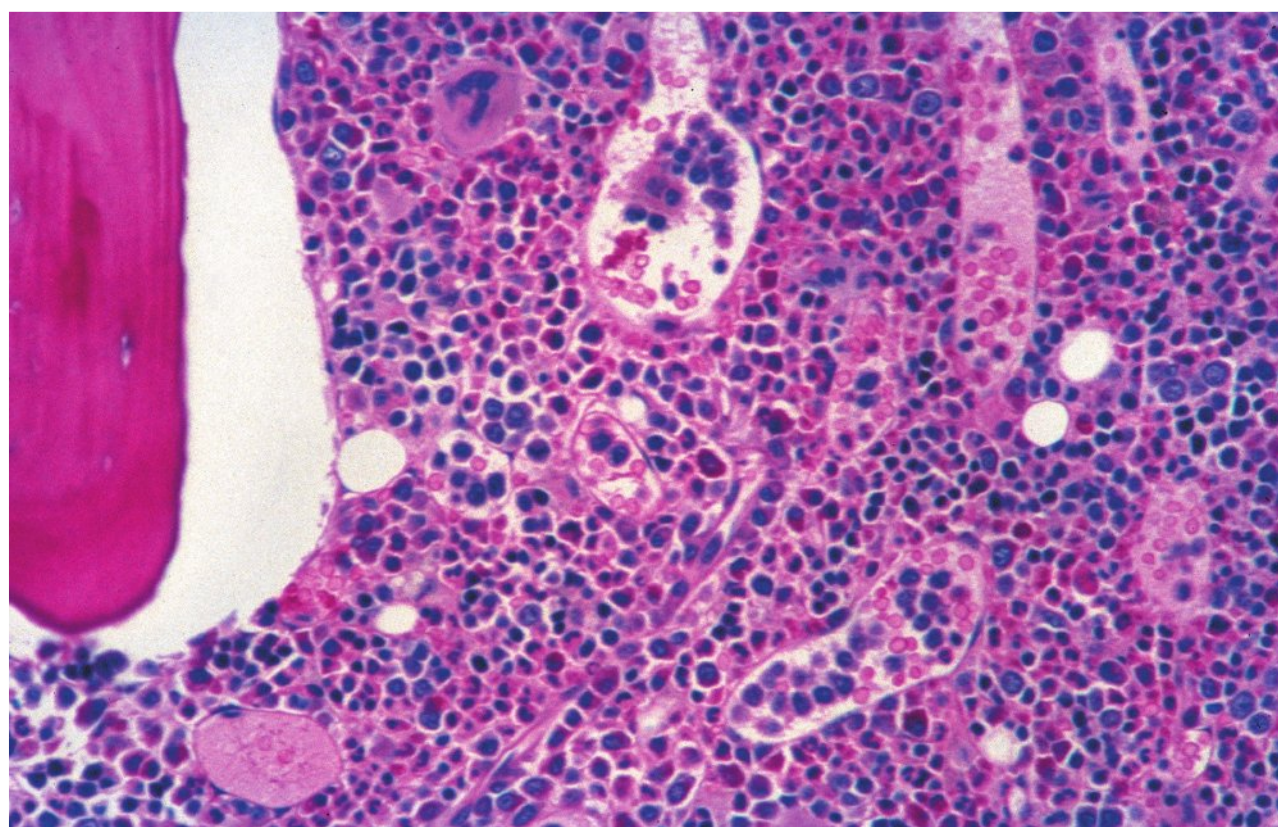


FIGURE 6.40.4 Bone marrow biopsy reveals sinusoidal lymphoma cell infiltration. Hematoxylin and eosin, 40× magnification. (Courtesy of Dr. Judith Brody, North Shore University Hospital, originally from Sun T. Flow Cytometric Analysis of Hematologic Neoplasms. Lippincott Williams & Wilkins; 2002.)

The bone marrow infiltration is usually mild, so the lesion is frequently missed (5,6,18). Therefore, immunohistochemical stains with CD3 and other markers are usually needed to accentuate the tumor cells. The sinusoidal infiltration pattern is an important clue to the diagnosis and should lead to further immunologic studies (Fig. 6.40.4).

Lymph nodes, as a rule, are not affected by this tumor (Fig. 6.40.5). Occasionally, the hilar lymph node of the spleen may show mild tumor cell infiltration.

The peripheral blood is usually devoid of lymphoma cells. However, a leukemic picture can be demonstrated in the late stage occasionally (3,19). A recent study demonstrated that the TCRab/CD3 to TCRgd/CD3 ratio in both peripheral blood and spleen suspension were significantly lower in the neoplastic gd T cells than the normal gd T cells (20). Therefore, this ratio can be used to detect

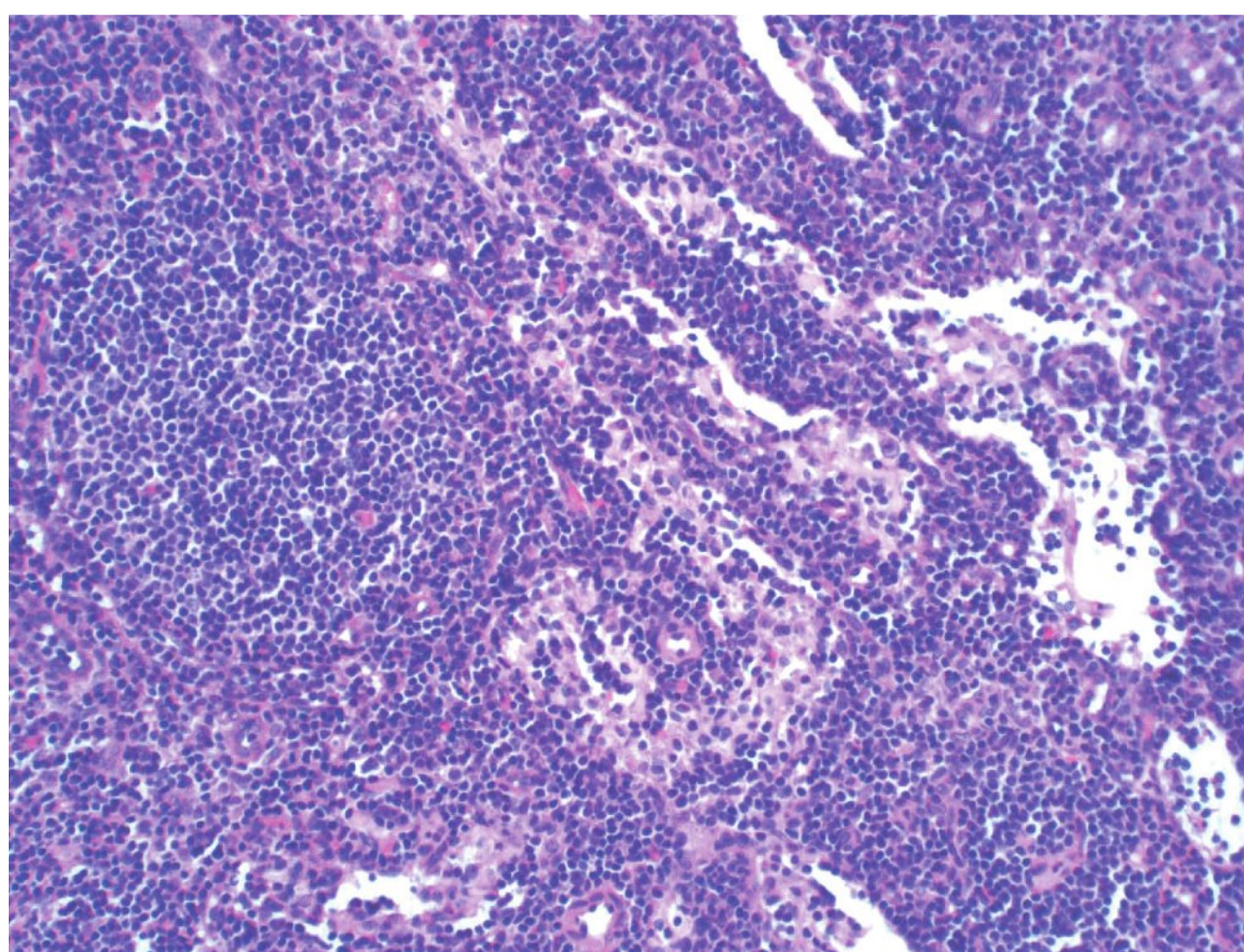


FIGURE 6.40.5 Lymph node from a patient with HSTCL shows only sinusoidal histiocytosis. No tumor cells are seen in the node. Hematoxylin and eosin, 20× magnification.

TABLE 6.40.1

Characteristic Morphologic Features of Hepatosplenic $\gamma\delta$ T-Cell Lymphoma

Histologic pattern	Sinusoidal infiltration of tumor cells in the spleen, liver, and bone marrow
Cytology	Monomorphic medium-sized tumor cells with slightly irregular nuclei and abundant cytoplasm
Specific feature	Sinusoidal infiltration in the spleen, liver, and bone marrow without lymph node involvement

the leukemic cells even when they show no morphologic atypia. The common hematologic findings are marked thrombocytopenia accompanied by certain degrees of anemia and leukopenia (1–3). The diagnostic morphologic features of HSTCL are summarized in Table 6.40.1.

Immunophenotype

Because there are so many characteristic morphologic features, a constellation of these findings may reach a reasonable diagnosis. However, recent efforts have been made to diagnose the disease by bone marrow biopsy alone to avoid splenectomy and liver biopsy (3). As bone marrow is usually mildly involved without any pathognomonic features, immunophenotyping becomes most important for the diagnosis.

Antibodies bF1 and TCRd1 can be used to identify ab T cells and gd T cells, respectively. Therefore, in cases of HSTCL, the immunophenotype is bF1–/TCRd1+. However, during progression, gdTCR may become lost, leading to a “TCR-silent” phenotype (bF1–/TCRd1–) (1). For the TCRd-variable region, most studies show that the tumor cells are positive only for Vd1, and not for Vd2 (2,4,21,22). Because Vd1 has a strong affinity to the splenic tissue, HSTCL always involves the spleen. However, only bF1 is applicable to paraffin sections. If bF1 stain is negative, the case is assumed to be a gd T-cell lymphoma.

T-cell subset studies may help, as gd T cells usually show a double-negative phenotype (CD3+, CD4–, CD8–) (Fig. 6.40.6), whereas ab T cells are either CD4+ or CD8+. However, a CD3+, CD4–, CD8+ phenotype can be seen in rare cases (Figs. 6.40.7 and 6.40.8) (14). One of the exceptions is intestinal gd T-cell lymphoma, in which CD8 is usually predominant (2). In HSTCL, CD2 is consistently positive, CD5 is characteristically negative, and CD7 can be positive or negative (14).

In addition to T-cell markers, the NK-cell and cytotoxic markers are important to establish the diagnosis. Among the NK cell-associated antigens, CD56, CD16, and CD11c are frequently positive, but CD57 is consistently negative (4). As TIA-1 (a cytotoxicity-associated molecule)

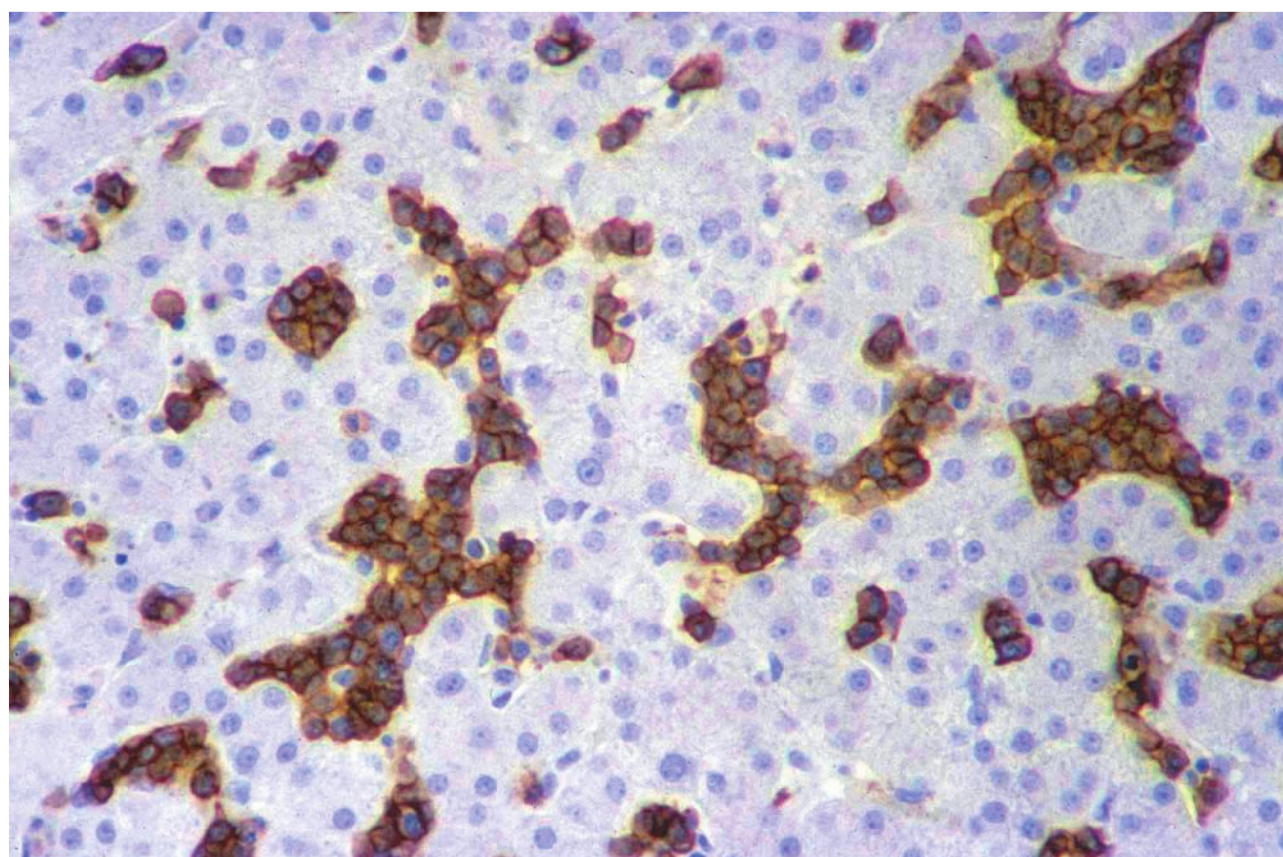


FIGURE 6.40.6 Liver biopsy reveals positive staining of the tumor cells with CD3 antibody. Immunoperoxidase, 40× magnification.

(Fig. 6.40.9) has been demonstrated in the tumor cells of HSTCL, the neoplastic gd T cells are considered cytotoxic T cells. However, other cytotoxic molecules such as perforin and granzyme B are frequently negative; HSTCL cells are, therefore, suggested by some authors to be inactivated cytotoxic T cells (1,2,23). Others consider HSTCL cells composed of a heterogeneous population of both activated and inactivated cytotoxic cells because perforin and granzyme B can be detected in some cases (2). Non-HSTCL gd T-cell lymphomas have an activated cytotoxic phenotype (expression of TIA-1, granzyme B, and perforin) and are most likely a heterogeneous group of diseases (14). Besides the T-cell subset, the immunophenotype of hepatosplenic ab T-cell lymphoma is similar to its gd T-cell counterpart (10–12).

Comparison of Flow Cytometry and Immunohistochemistry

For the diagnosis of HSTCL, immunohistochemistry is more useful than flow cytometry. The former can highlight

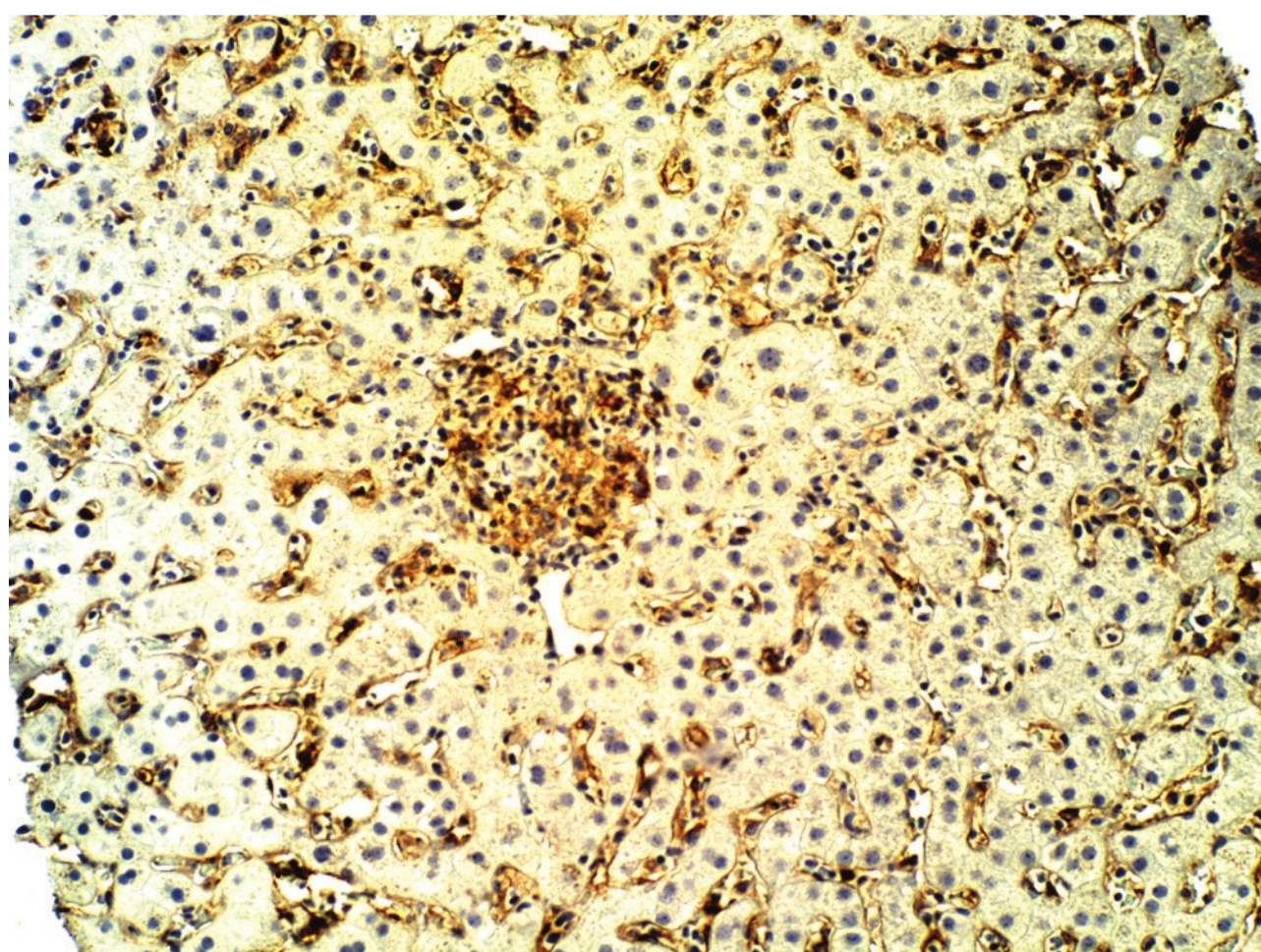


FIGURE 6.40.7 Liver biopsy reveals negative staining of the tumor cells with CD4 antibody. Immunoperoxidase, 20× magnification.

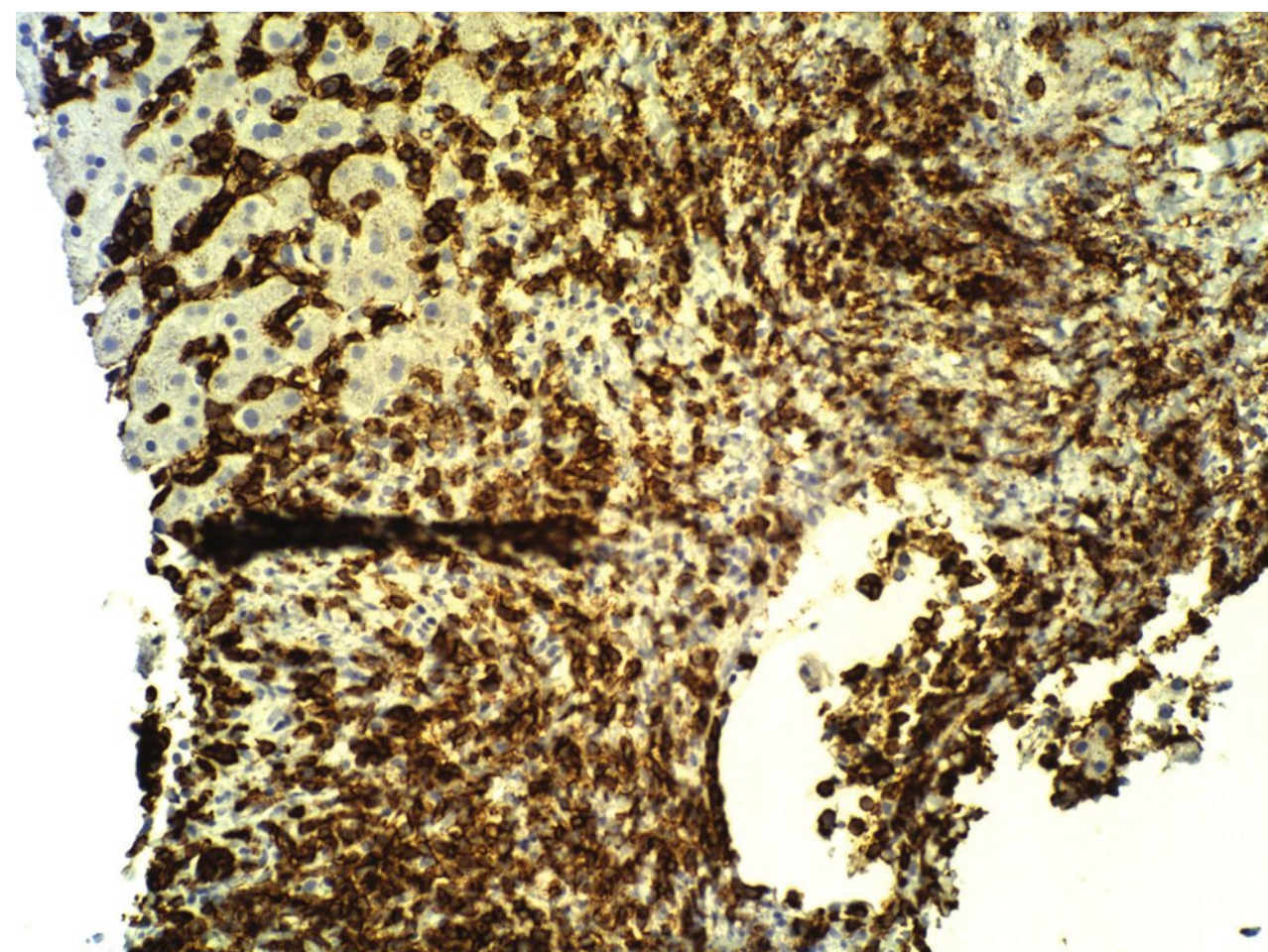


FIGURE 6.40.8 Liver biopsy shows positive staining of the tumor cells with CD8 antibody. Immunoperoxidase, 20× magnification.

the tumor cells and is particularly helpful in bone marrow biopsy, where tumor cell infiltration is usually mild. When frozen tissue is available, immunohistochemical stains can distinguish ab T cells from gd T cells. When necessary, immunohistochemical stains can also be used to identify Vd1 and Vd2 subtypes.

Molecular Genetics

As expected, HSTCL of gd T-cell origin usually shows rearrangement of both TCRg- and TCRd-chain genes. However, TCRb-chain gene rearrangement is also frequently detected in gd T-cell lymphomas. It seems that gd lineage commitment does not exclude TCRb-chain gene rearrangements, nor do TCRb gene rearrangements irrevocably incite the cell to ab TCR expression (2). On the other hand, TCRg and TCRd rearrangement can also be seen in ab T cells and the corresponding lymphomas (10,21). As mentioned before,

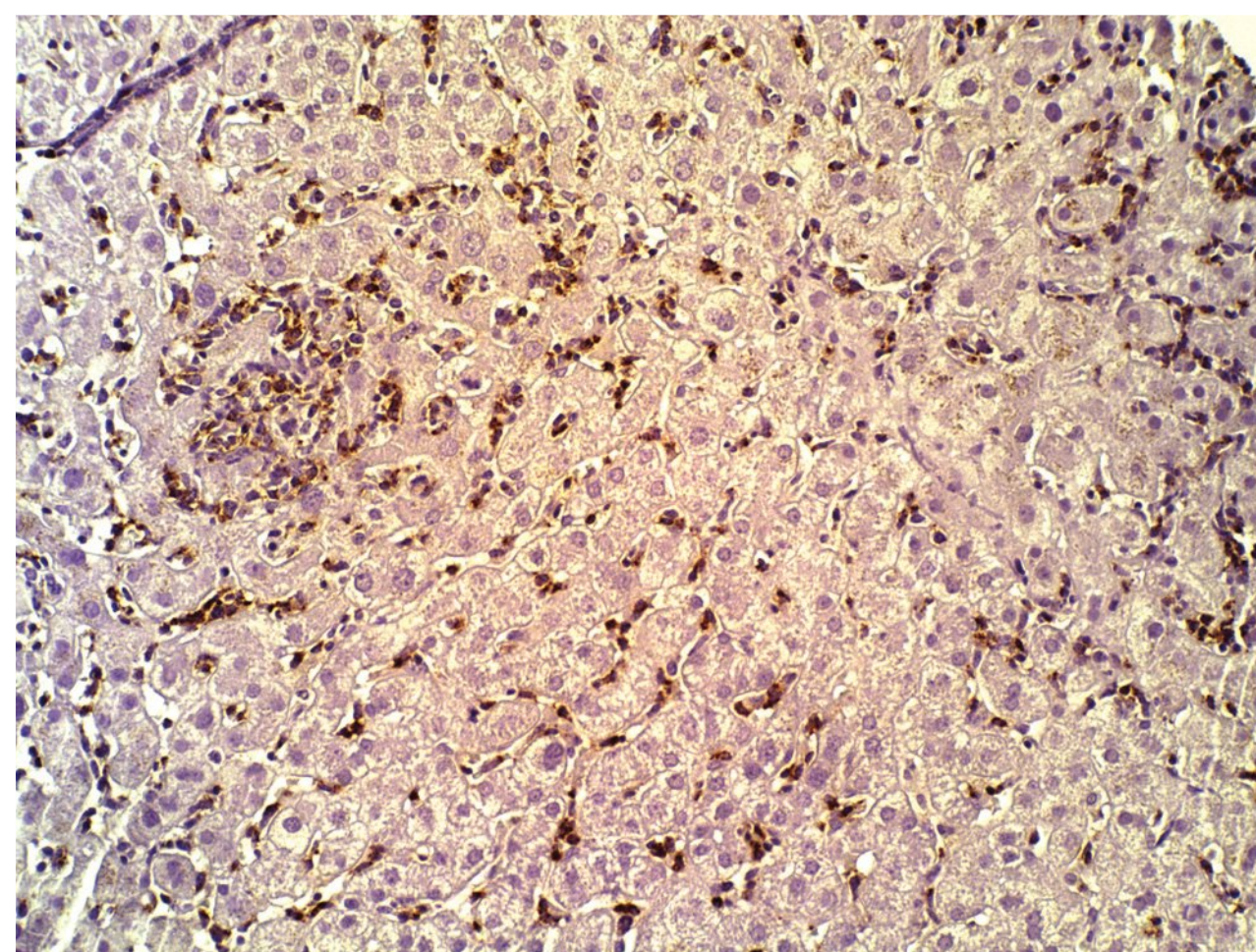


FIGURE 6.40.9 Liver biopsy reveals positive staining of the tumor cells with TIA-1 antibody. 20× magnification.

HSTCL usually expresses Vd1 protein, and the Vd1 gene can also be demonstrated by gene rearrangement analysis using the Vd1 probe (21).

Isochromosome 7q [i(7)(q10)] is the most common cytogenetic abnormality (2,4,24–27). The formation of isochromosome 7q is the result of losing one copy of the TCRg gene (expressed on 7p15) and duplicating the TCRb gene (expressed on 7q35) (27). Whereas this abnormality is also demonstrated in other hematologic malignancies (such as acute myeloid leukemia, acute lymphoblastic leukemia, prolymphocytic leukemia, and lymphoblastic lymphoma), its presence in those diseases is usually considered to be a secondary aberration associated with tumor progression (1,4,6). In HSTCL, isochromosome 7q is considered a primary aberration whether it is the sole abnormality or associated with other abnormal karyotypes.

The second most common chromosomal abnormality in HSTCL is trisomy 8, which is usually seen in acute myeloid leukemia (27). In a review of the literature, 12 of 19 reported cases of HSTCL had trisomy 8, and 11 of these cases presented with concomitant i(7)(q10) (27). Because trisomy 8 is frequently coexistent with isochromosome 7q and often present at the time of disease progression, it is considered to be a secondary event.

Loss of Y chromosome is the third common abnormality in HSTCL. However, this abnormality has been described as an age-related phenomenon in healthy men; therefore, its significance in pathogenesis of HSTCL is unclear. Other occasionally reported nonrandom chromosomal aberrations include t(7;9)(p15;q13), t(7;21)(q11.2;p11.2), t(13q;14q), -11, -22, and 14p+ with t(1;14)(q21;p13) (2,4,21,24,27).

In most reports, molecular identification of viral genomes usually showed negative results (15,26,28). The Epstein–Barr virus (EBV) genome by in situ hybridization and polymerase chain reaction has proven to be negative in many cases (28). Viral studies of human T-cell leukemia virus type 1, human immunodeficiency virus, human herpesvirus-6, and human herpesvirus-8 have also been negative (18,28). However, EBV-encoded small RNAs and EBV genomes have been identified in a nodal gd T-cell lymphoma (29) and in two Japanese cases of HSTCL (23).

In the current case, the lymph node biopsy was unremarkable. The sections from the splenectomy specimen, liver biopsy, and bone marrow biopsy showed extensive sinusoidal infiltration by medium-sized atypical lymphoid cells, mimicking malignant histiocytosis (30). However, both monocyte-histiocyte markers (CD11c and CD14) used in this study showed a low percentage of positive cells, a result that did not support the diagnosis of histiocytosis. In contrast, the high CD3-positive cell count with the concomitant loss of CD5 and CD7 in this T-cell population is consistent with a T-cell malignancy.

The diagnosis was substantiated by immunostaining the liver and spleen specimens. The immunoperoxidase stains revealed negative reactions to antibodies against cytokeratin, B-cell antigens (CD74, CDw75, and CD20), CD30, and CD15 but positive reactions to T-cell antibodies, UCHL1 (CD45RO), MT1 (CD43), and Leu 22 (CD43). The T-cell subset study showed the absence of CD4 and CD8

TABLE 6.40.2

Salient Features for Laboratory Diagnosis of HSTCL

Major phenotype: CD3+, CD4–, CD8–, TCRgd+, TCRab–

Minor phenotype: CD3+, CD4–, CD8+, TCRgd+, TCRab–

Selective loss of CD5 and/or CD7 (some cases)

Presence of NK markers: CD16, CD56, CD11c

TCR d–chain variable region: Vd1+ Vd2–

Cytotoxicity-associated molecules: TIA-1+, perforin±, granzyme B±

Monoclonal TCR g/d–chain gene rearrangement

TCR, T-cell receptor; NK, natural killer; TIA-1, T-cell intracellular antigen-1.

markers, which is characteristic of HSTCL. The pattern of sinusoidal infiltration of the spleen, liver, and bone marrow and the absence of lymph node involvement are certainly consistent with HSTCL. Our case did not show TCR gene rearrangement because a TCRg or a TCRd gene probe was not used. The salient features for laboratory diagnosis of HSTCL are summarized in Table 6.40.2.

Clinical Manifestations

The clinical features of patients with HSTCL are characterized by hepatosplenomegaly without lymphadenopathy. Bone marrow is often involved at diagnosis; therefore, patients are invariably in stage 4 at presentation (2). For this reason, bone marrow examination should be the first step for the diagnosis of HSTCL. In most circumstances, a diagnostic bone marrow may prevent further invasive procedures such as splenectomy and liver biopsy.

Lymphoma cells are seldom detected in the peripheral blood until the terminal stage, but pancytopenia is a constant feature in most patients and thrombocytopenia is most striking (5). The mechanism of pancytopenia may be associated with hypersplenism and/or the release of cytokines, such as interferon-γ by neoplastic gd T cells (3).

In a small group of patients, gd T-cell lymphoma involved mainly skin or mucosa at various anatomic sites, including the gastrointestinal and respiratory tracts (31). Rarely, lymphadenopathy is the initial clinical presentation, but spleen, liver, and bone marrow involvement appears at a later stage (22). In the 2008 WHO classification, primary cutaneous gd T-cell lymphoma is categorized as a separate entity (17).

Most patients are young (15 to 32 years at diagnosis) and predominantly male (5). A few pediatric cases have also been reported (27,32). Individuals usually present with pronounced clinical symptoms, including fatigue, fever, sweats, and abdominal pain (8,26). Hemophagocytic syndrome is an infrequent but serious complication, which often precipitates a rapidly downhill clinical course (2).

Many HSTCL cases have a condition of chronic immunosuppression or prolonged antigenic stimulation that includes transplantation, chemotherapy, hypogammaglobulinemia, T-cell deficiency, human T-cell leukemia virus type 1 infection, cytomegalovirus retinitis, and others (1,2,22,28,29). These predisposing factors are even more frequently encountered in nodal gd T-cell lymphoma or gd T-cell lymphoma involving the skin or mucosa. The above conditions may induce proliferation of gd T cells, and an additional transforming event, such as the acquisition of isochromosome 7, may finally lead to irreversible growth of the malignant clone (2,3).

In recent years, accumulated evidence suggests the association of the use of TNF blockers, particularly infliximab, in the treatment of inflammatory bowel disease in young patients and the development of HSTCL (16,33). Smaller numbers of HSTCL cases have been reported in association with other immunomodulating agents, such as azathioprine and mercaptopurine, for the treatment of inflammatory bowel disease (16,33).

A recently reported case of HSTCL developed during pregnancy (9). The authors hypothesized that this might be related to decreased immunity or hormonal imbalance during pregnancy. An interesting association is that the pregnant uterus and the decidual lymphocytes contain high concentrations of perforin, which is one of the cytotoxic molecules expressed by the gd T cells (9,10).

HSTCL is an aggressive lymphoma. Patients may respond to chemotherapy initially, but most cases show relapse after a few months to years (4). Splenectomy may induce elevated red blood cells and platelet counts, but it does not change the course of the disease (2). The median survival for HSTCL is 12 to 18 months, with a range of 3 to 42 months (1,4). When patients have blastic large-cell transformation, they usually die within a few months (4).

REFERENCES

- Gaulard P, Belhadj K, Reyes F. gd T-cell lymphoma. *Semin Hematol*. 2003;40:233–243.
- de Wolf-Peeters C, Achten R. gd T-cell lymphomas: a homogeneous entity? *Histopathology*. 2000;36:294–305.
- Belhadj K, Reyes F, Farcet JP, et al. Hepatosplenic gd T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. *Blood*. 2003;102:4260–4269.
- Salhany KE, Feldman M, Kahn MJ, et al. Hepatosplenic gd T-cell lymphoma: ultrastructural, immunophenotypic, and functional evidence for cytotoxic T lymphocyte differentiation. *Hum Pathol*. 1997;28:674–685.
- Cooke CB, Krenacs I, Stetler-Stevenson M, et al. Hepatosplenic T-cell lymphoma: a distinct clinicopathologic entity of cytotoxic gd T-cell origin. *Blood*. 1996;88:4265–4274.
- Weirich G, Sandherr M, Felibaum C, et al. Molecular evidence of bone marrow involvement in advanced case of Tgd lymphoma with secondary myelofibrosis. *Hum Pathol*. 1998;29: 761–765.
- Weidmann E. Hepatosplenic T cell lymphoma. A review on 45 cases since the first report describing the disease as a distinct lymphoma entity in 1990. *Leukemia*. 2000;14:991–997.
- Farcet JP, Gaulard P, Marolleau JP, et al. Hepatosplenic T-cell lymphoma: sinusal/sinusoidal localization of malignant cells expression the T-cell receptor gd. *Blood*. 1990;75:2213–2219.
- Niitsu N, Kohri M, Togano T, et al. Development of hepatosplenic gd T-cell lymphoma with pancytopenia during early pregnancy: a case report and review of the literature. *Eur J Haematol*. 2004;73:367–371.
- Falchook GS, Vega F, Dang NH, et al. Hepatosplenic gamma-delta T-cell lymphoma: clinicopathological features and treatment. *Ann Oncol*. 2009;20:1080–1085.
- Suarez F, Wlodarska I, Rigal-Huguet F, et al. Hepatosplenic ab T-cell lymphoma: an unusual case with clinical, histologic and cytogenetic features of gd hepatosplenic T-cell lymphoma. *Am J Surg Pathol*. 2000;24:1027–1032.
- Lai R, Larratt LM, Etches W, et al. T-cell lymphoma of a b lineage in a 16-year-old boy presenting with hemolytic anemia and thrombocytopenia. *Am J Surg Pathol*. 2000;23:459–463.
- Macon WR, Levy NB, Kurtin PJ, et al. Hepatosplenic ab T-cell lymphomas: a report of 14 cases and comparison with hepatosplenic gd T-cell lymphoma. *Am J Surg Pathol*. 2001;25: 285–296.
- Vega F, Medeiros LJ, Gaulard P. Hepatosplenic and other gd T-cell lymphomas. *Am J Clin Pathol*. 2007;127:869–880.
- Jaffe ES, Ralfkiaer E. Hepatosplenic T-cell lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:210–211.
- Gaulard P, Jaffe ES, Krenacs L, et al. Hepatosplenic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:292–293.
- Gaulard P, Berti E, Willemze R, et al. Primary cutaneous peripheral T-cell lymphomas, rare subtypes. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:302–303.
- Wu H, Wasik MA, Prezybylski G, et al. Hepatosplenic gamma-delta T-cell lymphoma as a late-onset posttransplant lymphoproliferative disorder in renal transplant recipients. *Am J Clin Pathol*. 2000;113:487–496.
- Steurer M, Stauder R, Grunewald K, et al. Hepatosplenic gd-T-cell lymphoma with leukemic course after renal transplantation. *Hum Pathol*. 2002;33:253–258.
- Baseggio L, Berger F, Monneret G, et al. The expression of TCR-gd/CD3 complex in neoplastic gd T-cell. *Hematologica*. 2006;91:1717–1719.
- Mastovich S, Ratech H, Ware RE, et al. Hepatosplenic T-cell lymphoma: an unusual case of a gd T-cell lymphoma with a blast-like terminal transformation. *Hum Pathol*. 1994;25: 102–108.
- Charton-Bain MC, Broussier P, Bouabdallah R, et al. Variation in the histological pattern of nodal involvement by gamma/delta T-cell lymphoma. *Histopathology*. 2000;36:233–239.
- Ohshima K, Haraoka S, Harada N, et al. Hepatosplenic gd T-cell lymphoma: relation to Epstein-Barr virus and activated cytotoxic molecules. *Histopathology*. 2000;36:127–135.
- Wong KF, Chan JKC, Matutes E, et al. Hepatosplenic gd T-cell lymphoma: a distinctive aggressive lymphoma type. *Am J Surg Pathol*. 1995;19:718–726.
- Wang CC, Tien HF, Lin MT, et al. Consistent presence of isochromosome 7q in hepatosplenic T gamma/delta lymphoma: a new cytogenetic-clinicopathologic entity. *Genes Chromosomes Cancer*. 1995;12:161–164.

26. Jonveaux P, Daniel MT, Martel V, et al. Isochromosome 7q and trisomy 8 are consistent primary, non-random chromosomal abnormalities associated with hepatosplenic T gamma/delta lymphoma. *Leukemia*. 1996;10:1453–1455.
27. Rossbach HC, Chamizo W, Dumont DP, et al. Hepatosplenic g/d-cell lymphoma with isochromosome 7q, translocation t(7;21), and tetrasomy 8 in a 9-year-old girl. *J Pediatr Hematol Oncol*. 2002;24:154–157.
28. Francois A, Lesesve JF, Stamatoullas A, et al. Hepatosplenic gamma/delta T-cell lymphoma: a report of two cases in immunocompromised patients, associated with isochromosome 7q. *Am J Surg Pathol*. 1997;21:781–790.
29. Kagami Y, Nakamura S, Suzuki R, et al. A nodal gd T-cell lymphoma with an association of Epstein-Barr virus. *Am J Surg Pathol*. 1997;21:729–736.
30. Sun T, Brody J, Susin M, et al. Extranodal T-cell lymphoma mimicking malignant histiocytosis. *Am J Hematol*. 1990;35:269–274.
31. Arnulf B, Copie-Bergman C, Delfau-Larue MH, et al. Nonhepatosplenic gd T-cell lymphoma: a subset of cytotoxic lymphomas with mucosal or skin localization. *Blood*. 1988;91:1723–1731.
32. Garcia-Sanchez F, Menarguez J, Cristobal E, et al. Hepatosplenic gamma-delta T-cell malignant lymphoma: report of the first case in childhood, including molecular minimal residual disease follow-up. *Br J Haematol*. 1995;99:943–946.
33. Mackey AC, Green L, Leptak C, et al. Hepatosplenic T cell lymphoma associated with Infliximab use in young patients treated for inflammatory bowel disease: update. *J Ped Gastroenterol Nutr*. 2009;48:386–388.

CASE 41

Mycosis Fungoides and Sézary Syndrome

CASE HISTORY

A 72-year-old man presented with a 2-week history of a whole-body pruritic, erythematous rash with scaling as well as purpuric nodules on the right thigh. The patient also had increased fatigue, bilateral lower extremity swelling, and scrotal edema. He denied any trauma to the leg, or exposure to new medications, soaps, detergents, or perfumes.

A lower extremity ultrasound was negative for deep venous thrombosis. The nodule on the right thigh was aspirated, and Gram stain and cultures were negative. However, physical examination revealed posterior auricular, axillary, and inguinal lymphadenopathy.

A skin biopsy was performed that showed lymphocytic infiltration in the dermis and epidermis. The Oncology Service was consulted, and the oncologist recommended further examination of the peripheral blood and bone marrow. The peripheral blood showed a total leukocyte count of 10,000/mL with 26% neutrophils, 5% bands, and 55% lymphocytes. Many lymphocytes revealed cerebriform nuclei consistent with Sézary syndrome (SS). Atypical lymphocytes were also demonstrated in the bone marrow. A right axillary lymph node (LN) biopsy was subsequently performed that demonstrated total replacement of the normal architecture by the tumor cells. A computed tomography scan of the abdomen and pelvis showed mediastinal and abdominal lymphadenopathy.

The patient was treated with psoralen plus ultraviolet A (PUVA) and interferon- α , but he responded poorly. As the patient also had congestive heart failure, chronic renal insufficiency, and adult-onset diabetes mellitus, his condition deteriorated rapidly, and he died approximately 3 months after the diagnosis.

FLOW CYTOMETRY FINDINGS

Peripheral blood: CD2 94%, CD3 97%, CD3/CD4 94%, CD3/CD8 3%, CD5 96%, CD7 32%, CD19 1%, CD20 0%, k 1%, l 1%, CD14 0%, CD25 18%, CD30 3% (Fig. 6.41.1).

Bone marrow: CD3 94%, CD3/CD4 85%, CD3/CD8 10%, CD5 90%, CD7 12%, CD19 6%, CD25 0%.

LN biopsy: CD3 90%, CD3/CD4 88%, CD3/CD8 2%, CD5 90%, CD7 19%, CD19 9%, CD25 8%.

IMMUNOHISTOCHEMISTRY FINDINGS

The lymphoid infiltrate in the skin showed positive staining for CD3 and CD4, but negative staining for CD8 and CD20.

DISCUSSION

Mycosis fungoides (MFs) is a primary cutaneous T-cell lymphoma (CTCL) with a clinical presentation of patch, plaque, and tumor stages, and an epidermal and dermal skin infiltration by small- to medium-sized lymphoid cells with cerebriform nuclei (1–3). It is the most common CTCL, accounting for about one half of all primary cutaneous lymphomas (3). The incidence is increased in the United States, with about 0.2 cases per 100,000 population in 1973 to 0.4 cases per 100,000 population in 1984 (4). Thus, the absolute number of new cases is about 1,000 per year. In the earlier literature, SS was considered an erythrodermic leukemia variant of MF (1,4). However, the new classifications, including the World Health Organization (WHO)-European Organization for Research and Treatment of Cancer (EORTC) classification

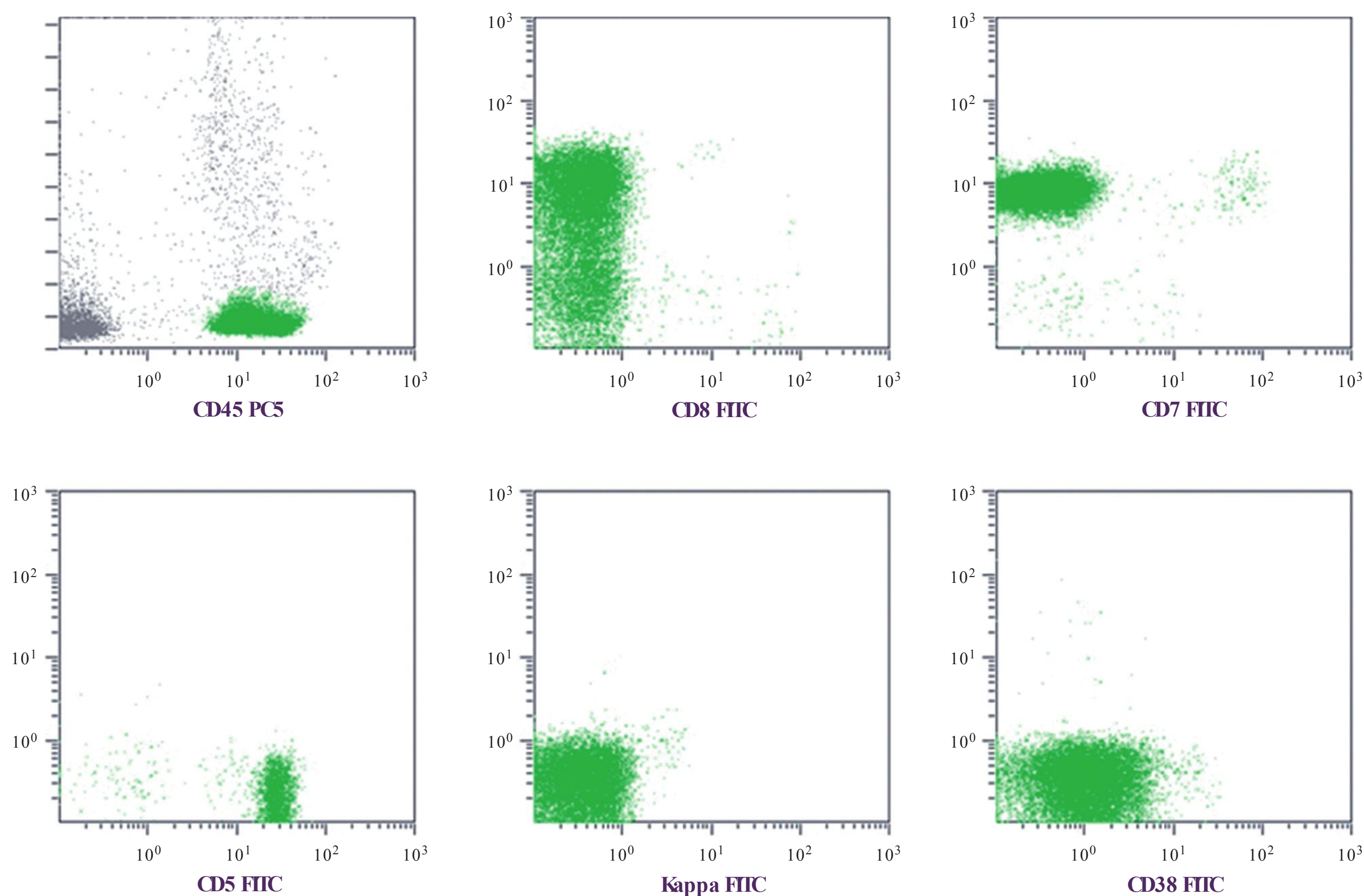


FIGURE 6.41.1 Flow cytometric histograms show positive for CD3, CD4, CD5, and CD38 (activated T-cell antigen), but negative for CD7, CD20, κ , and λ . SS, side scatter; PC5, phycoerythrin-cyanin 5; PE, phycoerythrin; FITC, fluorescein isothiocyanate; ECD, phycoerythrin-Texas-Red.

for cutaneous lymphomas, generally separate them into two distinct entities, partly because their prognoses are markedly different (2,5,6). If a case meets the criteria of SS, but is preceded by clinically typical MF, it is designated SS preceded by MF or secondary SS (7).

Morphology

The histologic features of MF/SS overlap with other cutaneous lymphomas and even some benign inflammatory conditions. Therefore, clinical correlation is of utmost importance in making a morphologic diagnosis. The histologic pattern varies in different clinical stages of MF and in SS (3,8). A patch lesion may reveal scanty to patchy lymphocytic infiltration in the upper dermis. It is usually perivascular rather than bandlike. Intraepidermal lymphoid infiltration (Pautrier microabscesses) is rare or absent. Cytologic atypia, if present, is minimal. The plaque lesion is characterized by a dense, bandlike infiltrate of atypical lymphoid cells in the upper dermis (Fig. 6.41.2). Pautrier microabscesses are often present. The atypical lymphoid cells show hyperchromatic and convoluted nuclei and scant cytoplasm. The deep dermis and subcutis are seldom involved. The tumor lesion, in contrast, shows a dense infiltrate of atypical lymphoid cells that may extend into the lower dermis and subcutis. At this stage, the tumor cells

increase in number and in size, so that a mixed population of small, medium-sized, and large cerebriform cells as well as blasts with prominent nucleoli is present (4). Epidermal infiltration, in contrast, gradually disappears.

The features that are considered most specific for MF include Pautrier microabscesses and the presence of lymphocytes within the epidermis that are larger than those within the dermis (5). The definition of Pautrier microabscesses by the EORTC is “sharply margined clusters of atypical lymphoid cells... that were closely opposed to one another with uniform cytologic features... with no plasma or fibrin deposition or significant cytopathic changes in the surrounding keratinocytes” (9). The presence of convoluted lymphocytes 7 to 9 mm in diameter, epidermal infiltration with single-haloed lymphocytes (lymphocytes surrounded with vacuoles) (Fig. 6.41.3), lining up of lymphocytes along the basal layer, and a lichenoid infiltrate that spares the dermal epidermal junction are also considered specific diagnostic features for MF (5,9,10).

The International Society for Cutaneous Lymphomas (ISCL) has classified erythrodermic CTCLs into three categories: SS, erythrodermic MF (secondary erythrodermic CTCL developed in patients with MF), and erythrodermic CTCL, not otherwise defined (11). In this classification, SS consists of one or more of the following features: (a) an absolute

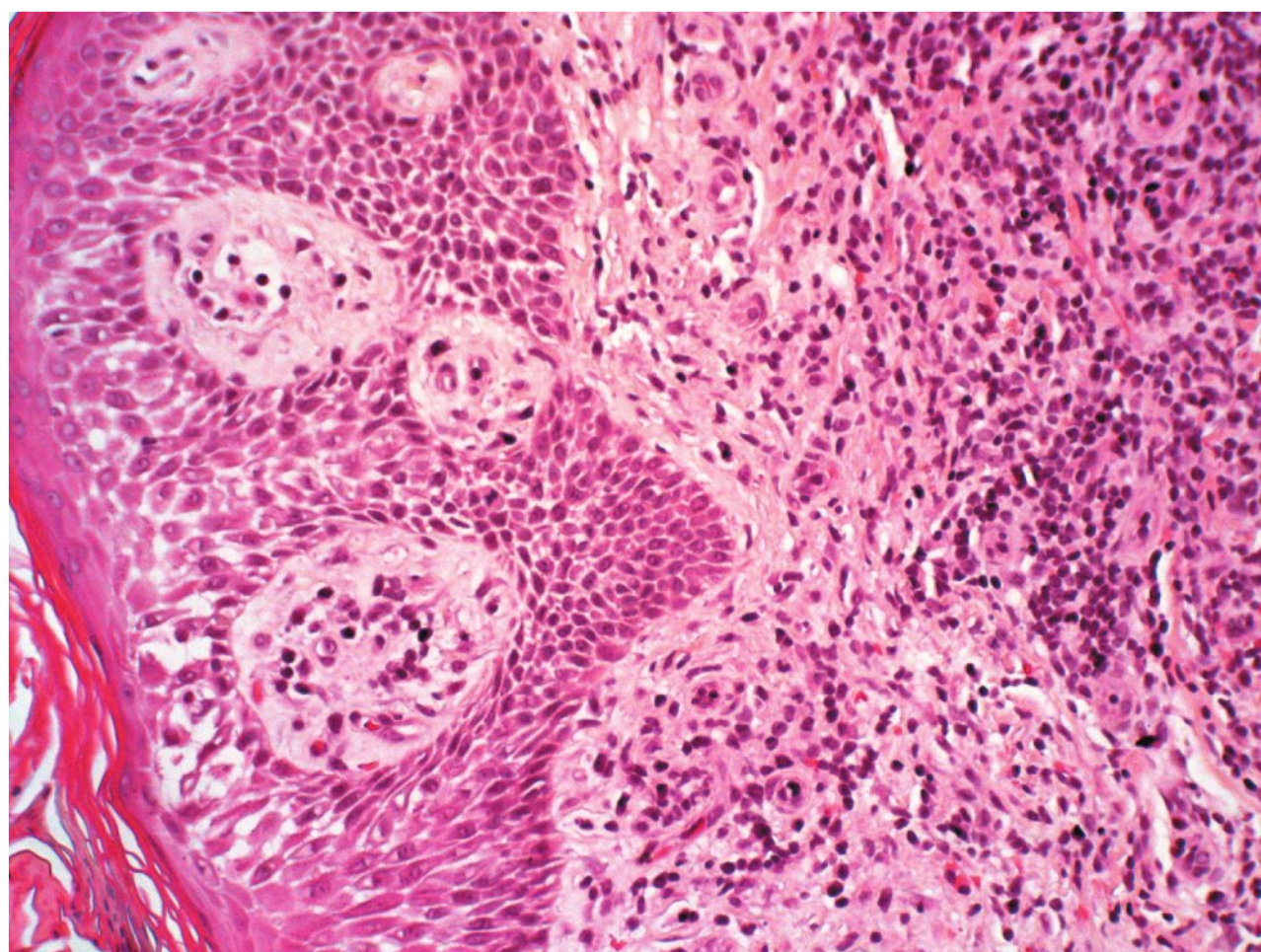


FIGURE 6.41.2 Skin biopsy from a patient with MFs shows a few Pautrier microabscesses in the epidermis as well as a bandlike infiltration in the upper dermis. Hematoxylin and eosin, 20× magnification.

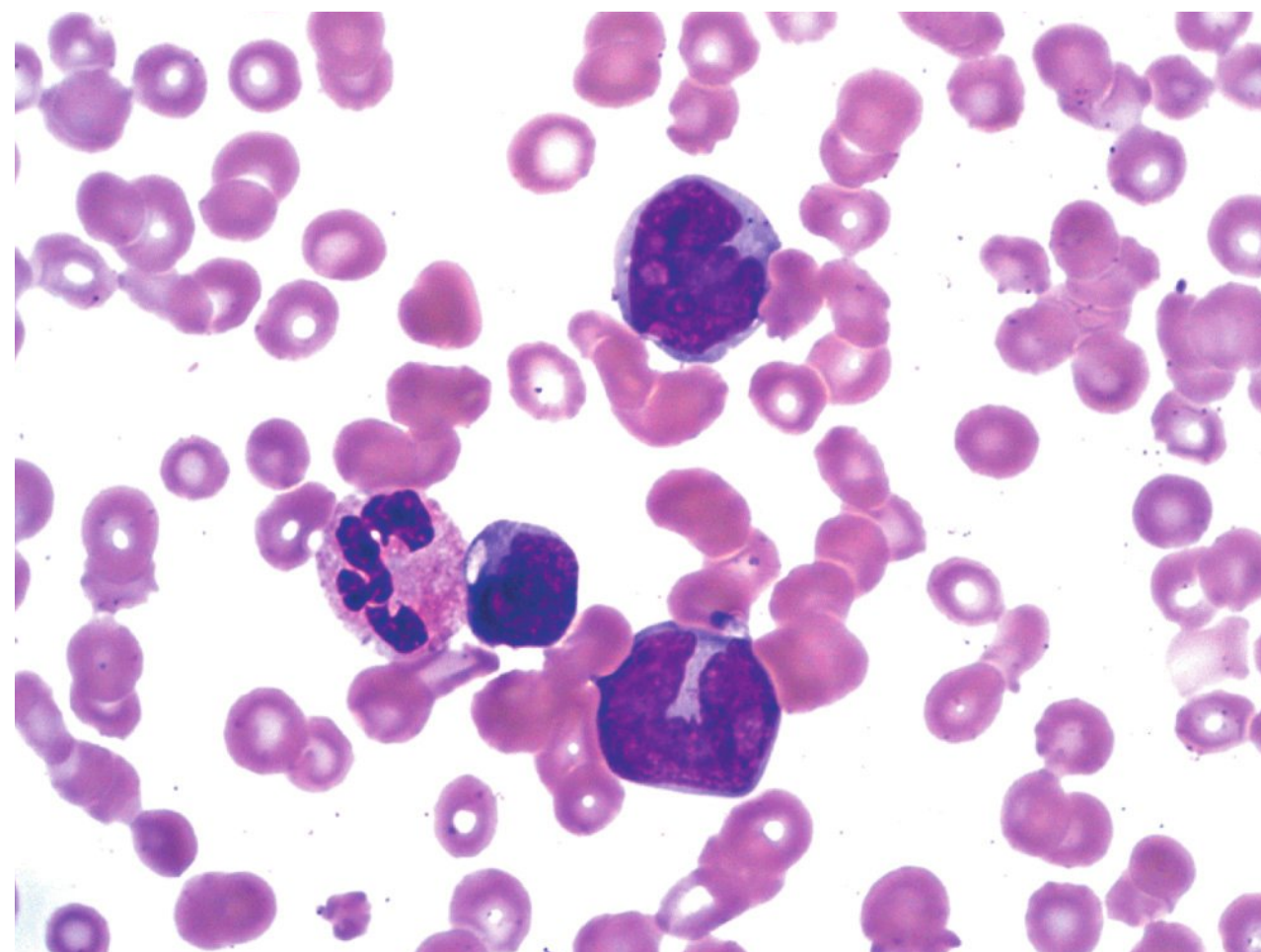


FIGURE 6.41.4 Peripheral blood smear from a case of SS reveals three Sézary cells and one neutrophil. Cell in the upper field shows typical cerebriform nucleus with prominent nucleoli. Wright–Giemsa, 100× magnification.

Sézary cell count of $\geq 1,000/\mu\text{L}$ (Fig. 6.41.4); (b) demonstration of immunophenotypic abnormalities: CD4/CD8 ratio of ≥ 10 or higher or aberrant loss of pan-T-cell markers by flow cytometry; and (c) demonstration of a T-cell clone in the peripheral blood by molecular or cytogenetic techniques (11).

Histologically, SS may differ from MF in more obvious monotonous cellular infiltration and less conspicuous epidermotropism (3). The cutaneous features may also be nonspecific in up to one third of cases (3). However, the LN usually shows effacement of the normal architecture with a dense, monotonous infiltrate of Sézary cells (3). In equivocal cases, ultrastructural morphometric measurement of the nuclear size and shape, chromatin distribution, and nuclear/cytoplasmic ratio are helpful in differential diagnosis (12). Sézary cells are positive for periodic acid-Schiff, tartrate-resistant

acid phosphatase, and b-glucuronidase, whereas normal lymphocytes are negative for these reactions (13).

The histologic features in the LN of MF/SS cases are most commonly those of dermatopathic lymphadenopathy (DL). Characteristically, the LN contains sinus histiocytosis, an abundance of pigment-laden macrophages, and various numbers of atypical lymphocytes depending on the stage of the disease (1). According to the degree of tumor cell involvement, a grading system divides the LN changes into four categories (14). LN1 shows DL with occasional atypical lymphocytes. LN2 is designated when clusters of <6 atypical lymphocytes are demonstrated. When >15 atypical cells are seen in clusters, it is graded LN3. When the normal architecture of the LN is partially or completely effaced, it becomes LN4 (Fig. 6.41.5). Currently, there are

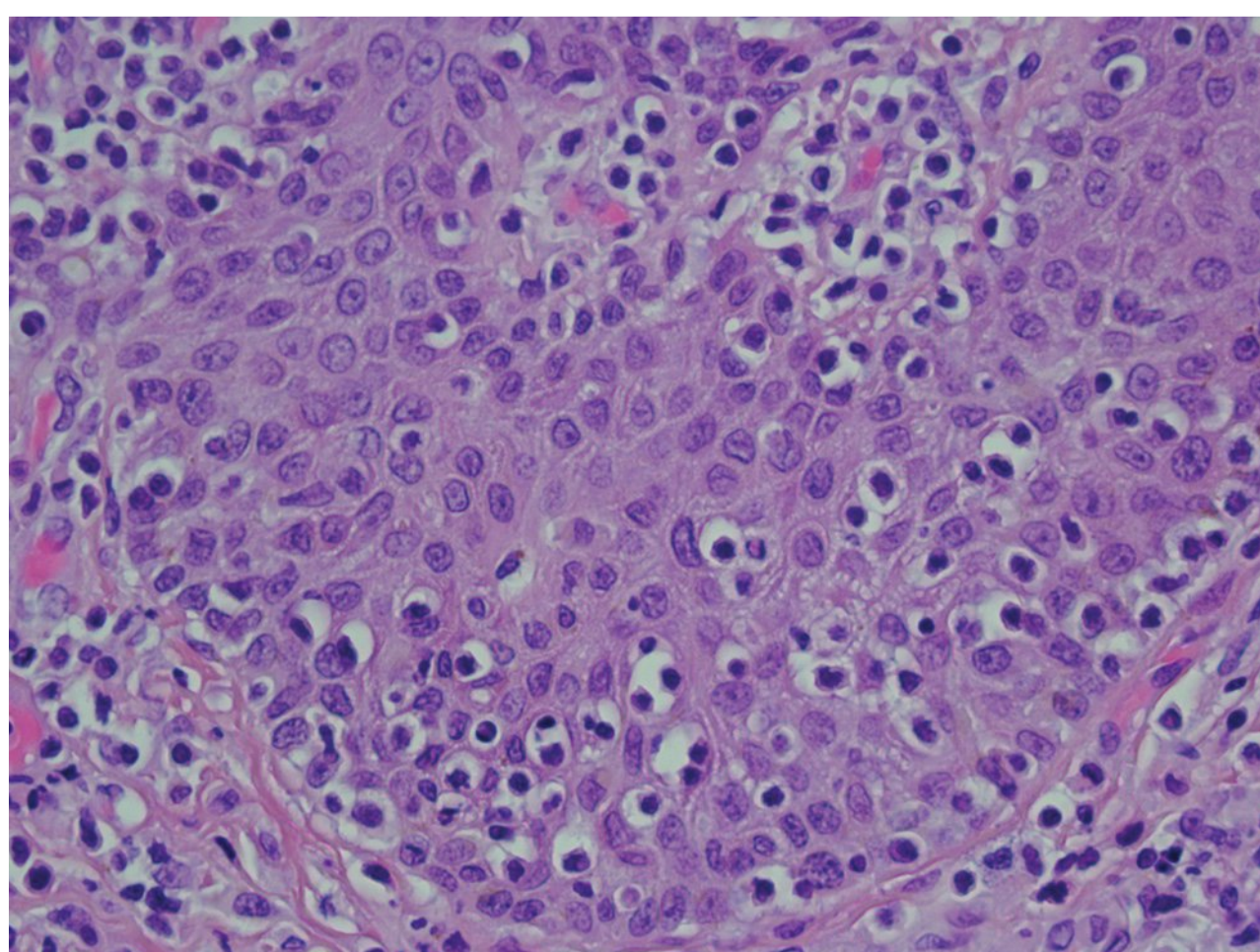


FIGURE 6.41.3 Skin biopsy from a patient with MFs shows many single haloed lymphocytes. Hematoxylin and eosin, 40× magnification.

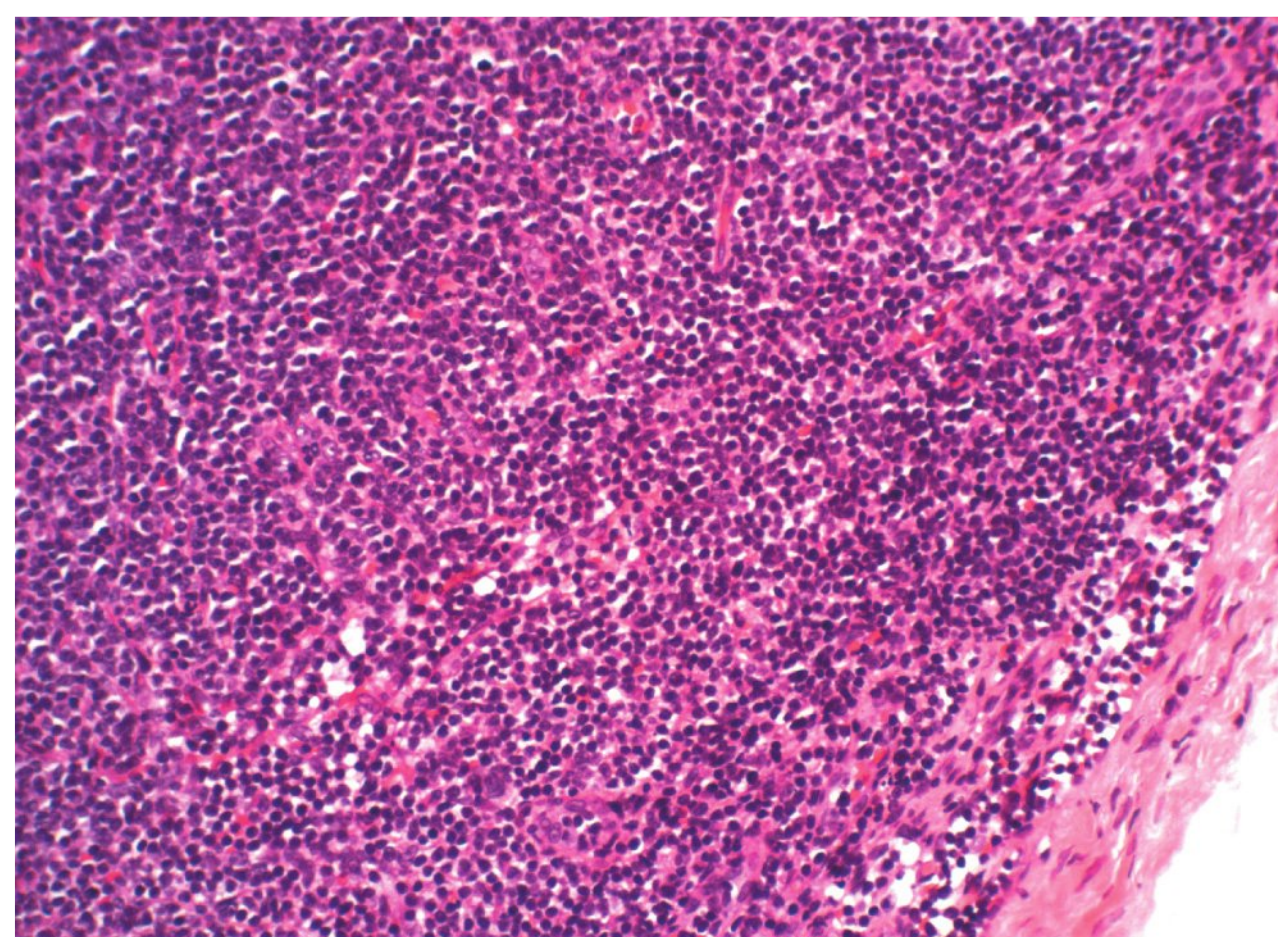


FIGURE 6.41.5 LN biopsy from a case of SS shows total effacement of the normal architecture by the lymphoid tumor cells. The lesion is considered to be in the LN4 grade. Hematoxylin and eosin, 20× magnification.

TABLE 6.41.1

Histopathologic Grading System of LN in MF/SS

ISCL/ EORTC	Dutch system	NCI/ VA system
N ₁	Grade 1: dermatopathic lymphadenopathy (DL)	LN ₀ : no atypical lymphocytes LN ₁ : occasional and isolated atypical lymphocytes LN ₂ : many atypical lymphocytes or in 3–6 cell clusters
N ₂	Grade 2: DL; early involvement by MF (presence of cerebriform nuclei >7.5 mm)	LN ₃ : aggregates of atypical lymphocytes; nodal architecture preserved
N ₃	Grade 3: partial effacement of LN architecture; many atypical cerebriform mononuclear cells	LN ₄ : partial/complete effacement of nodal architecture by atypical lymphocytes or frankly neoplastic cells
	Grade 4: complete effacement	

three major histopathologic grading systems for LNs in MF/SS, that is, the ISCL/EORTC, Dutch, and NCI/VA classifications (Table 6.41.1) (7).

There are several less common presentations of MF (6,15), but only three variants are recognized in the new WHO-EORTC classification (3). Folliculotropic MF is characterized by perifollicular and intrafollicular infiltrates of atypical lymphocytes without epidermotropism and with or without follicular mucinosis (15,16). Pagetoid reticulosis is characterized by the presence of localized patches or plaques with an intraepidermal proliferation of neoplastic T cells (3). The granulomatous slack skin shows MF features in the epidermis and papillary dermis and sarcoidal granuloma or granuloma annulare–like palisading granuloma in the reticular dermis. Lymphophagocytosis by the multinucleated giant cells is sometimes present (17).

Transformation of MF (T-MF) to a large T-cell lymphoma has been demonstrated in 8% to 55% of MF patients (18). The current definition of T-MF is the presence of >25% large cells in the infiltrate throughout the biopsy or presence of microscopic nodules of large cells (18,19). The major differential diagnosis is granulomatous MF in which the histiocyte/macrophages may mimic large cells (18). In case of doubt, a CD68 (PG-M1) stain should be performed to identify the histiocytes and/or macrophages. T-MF should also be distinguished from MF coexistent with a CD30+ lymphoproliferative disorder, such as anaplastic large-cell lymphoma or lymphomatoid papulosis (18). T-MF carries a poor prognosis, whereas primary cutaneous CD30+ lymphoproliferative disorders have a good prognosis.

In a review of 29 autopsies of MF patients, LN was involved in 37.9%; spleen, 34.5%; liver, 31.0%; lungs, 27.6%; bone marrow, 24.1%; and pleura, stomach, and kidneys, 17.2% each (20). Hepatic involvement by MF/SS usually manifests as nodular aggregates of tumor cells in the portal zones or the lobules, and bone-marrow involvement may show nodular aggregates or diffuse infiltrate by the

tumor cells (Fig. 6.41.6) (21). In general, bone-marrow infiltration is more frequently demonstrated in SS than in MF with limited skin disease (1). However, bone marrow can appear uninvolved even if significant numbers of circulating Sézary cells are detected (4).

Immunophenotype

Immunophenotyping is not very specific, but it is highly sensitive for the distinction of MF/SS from other non-neoplastic entities. Weiss et al. (22) found it helpful to distinguish DL from LNs with Sézary cell involvement. MF/SS cells are generally positive for CD2, CD3, CD4, and CD5 but are characteristically negative for CD7, CD8, CD1a, and terminal deoxynucleotidyl transferase (23,24).

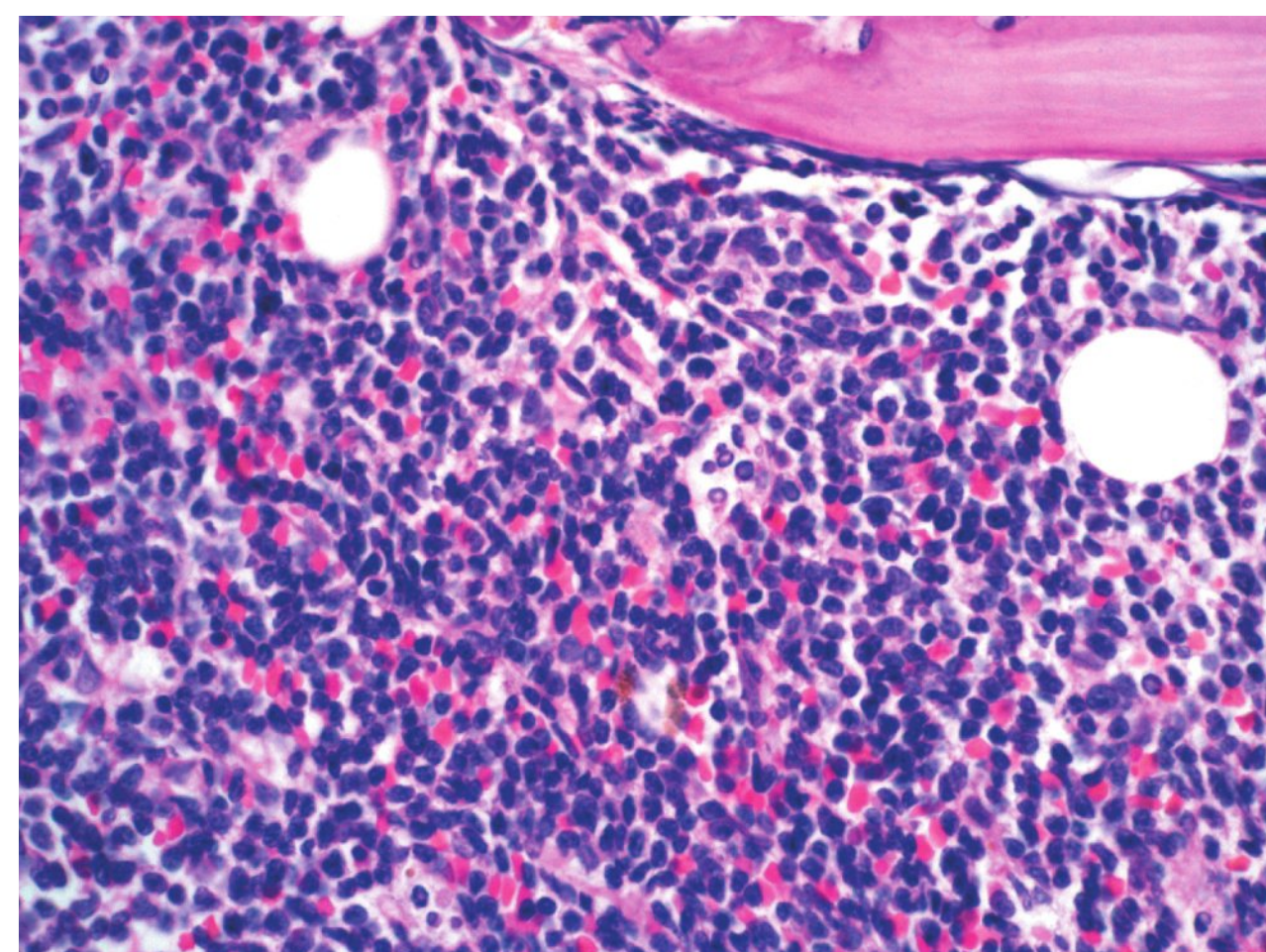


FIGURE 6.41.6 Bone-marrow biopsy from a patient with Sézary syndrome reveals extensive interstitial infiltration by the small tumor cells with irregular nuclei. Hematoxylin and eosin, 40× magnification.

In immunohistochemical staining, CD45RO is usually positive, which makes Sézary cells the memory helper T cells (CD4+, CD45RO+) (4). Suppressor T-cell type (CD8+) has been demonstrated occasionally in MF cases. However, such cases have the same clinical behavior and prognosis as CD4+ cases (3).

Although the absence of CD7 is an important finding in the diagnosis of MF/SS, CD7 is expressed in one third of MF patients (4). In the early stage of MF, the percentage of CD7 may be normal or only partly lost. Bergman et al. (25) found that when the percentage of CD7 was $\leq 36\%$ by manual counting on immunohistochemical-stained sections, it would be supportive for the diagnosis of CTCL rather than inflammatory skin disorders. Another study used 50% as the cutoff point to define deficiency of CD7 and CD62 ligands (CD62L; L-selectin) in MF/SS (21). The loss of CD7 and CD62L on T-cells is more helpful in identifying LN than skin involvement by MF/SS, because this phenomenon is seldom demonstrated in reactive LNs (21). Nevertheless, CD7 loss cannot reliably distinguish CTCL from benign dermatoses in cases with indetermined histology (25).

In some cases, the loss of CD2, CD3, or CD5 is also helpful in identifying neoplastic infiltration (24). Such deficiency is detected in the intraepidermal T cells but not intradermal T cells in 10% of cases of MF/SS (21). This discordance of immunophenotype between the epidermal and dermal T cells can be used to distinguish MF/SS from other skin lesions.

Michie et al. (26) studied the T-cell receptor (TCR) antigens in MF and found that early cases showed the normal CD3+, TCR β +, TCR γ - phenotype, but abnormal CD3/TCR β antigen expression was seen in 50% of tumor stage MF cases (26). The use of TCR V β chain antibodies in flow cytometry greatly facilitates the identification of T-cell clonality. A study in M.D. Anderson Cancer Center successfully identified a clonal T-cell population in 60/82 MF and 6/6 SS patients, while the results were negative in 10 healthy donors and 18 control patients (27). By staining with a panel of monoclonal antibodies directed against a specific TCR-V β subfamilies, it was found by Campbell et al. (28) that SS is a malignancy of control memory T cells (CCR7+, L-selectin+, CD27+), while MF is a malignancy of skin resident effector memory T cells (CCR4+, CLA+).

There are several rare, but clinically important markers. CD25 is negative in at least half the cases of MF (4); its expression may predict large-cell lymphoma transformation and poor prognosis (29). The expression of cytotoxic proteins (T-cell intracellular antigen-1 [TIA-1] and granzyme B) has been found to be increased with progression from plaque stage to tumor stage in CD4+ MF patients (30). A few cases with the immunophenotype of CD4-, CD8+, CD56+, TIA+, granzyme B+ have also been reported (31). In addition, there are two new markers, CD158k and T-plastin, which are reported to be specific for Sézary cells, but only limited studies are available (32,33). T-plastin (PL53) gene expression may also help to distinguish SS from MF and inflammatory skin diseases (34). A recent study also demonstrated the value of losing CD26 (CD4+, CD26-) in the diagnosis of MF/SS (35).

The epidermotropism of malignant T cells in MF/SS is an intriguing phenomenon involving lymphocyte-keratinocyte interaction. An earlier study suggested that the lymphocyte function-associated antigen 1 of the T lymphocyte may bind to the intercellular adhesion molecule 1, which forms the basis of epidermotropism (36). A current study presented a more complicated feature, which includes the interactions of lymphoma-cell integrin α_{E7} , CC chemokine receptor (CCR)4, and the CD4 TCR complex with E-cadherin, CCL22, and major histocompatibility complex class II molecules of the Langerhan cell, respectively (37). The cutaneous lymphocyte antigen and the CCR4 on the lymphoma cell surface are responsible for extravasation from the dermal capillaries (37).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometric analysis may demonstrate the selective loss of pan-T-cell antigens, particularly CD7, and the alteration of CD4/CD8 ratio that are essential for the diagnosis of MF/SS. However, this immunophenotype can be seen in other peripheral T-cell lymphomas as well. Immunohistochemistry has the advantage of demonstrating separate immunophenotypes in intraepidermal and intradermal T cells, and can be helpful to distinguish MF/SS from other skin lesions. Nevertheless, the calculation of CD4/CD8 ratio in immunohistochemistry is not as accurate as in flow cytometry.

In the current case, the patient had generalized pruritic, erythematous skin lesion, and generalized lymphadenopathy. Skin biopsy showed intraepidermal Pautrier microabscesses and bandlike infiltration with cerebriform lymphocytes in the upper dermis. Lymphoid cells with the same morphology were found in the peripheral blood, bone marrow, and LNs. The LNs showed a total effacement of the normal architecture by the tumor cells. Immunohistochemical staining of the skin biopsy identified a helper T-cell phenotype (CD3+, CD4+, CD8-). Flow cytometry, in addition, demonstrated the selective loss of CD7 in the tumor cells in the peripheral blood, bone marrow, and LNs. In conclusion, this patient had disseminated SS that led to his prompt death. Based on the tumor, node, metastasis, blood (TUMB) staging system, the patient was in stage IVa: T₄, N₃, M₀, B₁.

Molecular Genetics

Molecular biological technique is the most sensitive means used to identify MF/SS cells and distinguish them from reactive lymphoid cells in peripheral blood, LNs, and visceral organs (38). Whittaker et al. (39) detected TCR gene rearrangement in the skin lesion and the peripheral blood in some patients with MF of both early and late stages. However, five patients in early stages showed only germline configuration, suggesting that some cases may start as a polyclonal lymphoproliferative disorder. Six patients with SS also showed no TCR gene rearrangement, but only one of the six patients died of an unrelated cause. On the contrary, five of eight SS patients with clonal disease died during the period of observation (39). Another interesting finding was that a dual TCR and heavy-chain gene

rearrangement was detected in 4 of 11 patients with SS, but none of the MF cases showed additional heavy-chain gene rearrangement (39).

In early lesions of MF (patch stage) in which the number of tumor cells is low, Southern blotting may not be sensitive enough to detect clonal T-cell population (4,40). Therefore, polymerase chain reaction, with the capability of amplifying DNA, has gradually replaced Southern blotting. The positive rate of polymerase chain reaction is about 50% to 80% in patients with patch and plaque stage disease (17), whereas it is 100% for those in the tumor stage and 83% in those in the erythroderma stage (41). For indeterminate lesions, the positive rate is only 16% (22). In contrast, the demonstration of a monoclonal band, particularly a low-density one, can be seen in benign T-cell proliferation as a result of local expansion of stimulated cells (4). Therefore, clinicopathologic correlation is still the gold standard for the diagnosis of MF/SS.

Clonal identification is probably more helpful for monitoring the disease than for initial diagnosis. Dereure et al. (42) found that clonal rearrangements in the skin disappeared in 8 of 13 patients who showed a complete clinical and histopathologic response, but not in 10 patients with histologically persistent disease. Flow cytometry, using TCR Vb 14 antibodies, has also been successfully applied to therapeutic monitoring (43).

Many cytogenetic abnormalities have been found in MF/SS, but recurrent specific aberrations have not been identified thus far. The most common karyotypic abnormalities include loss of chromosome 10, deletions of 1p and isochromosome 17q, additions of 17p and 19p, and translocations involving 1p, 10q, and 14q (44). Abnormalities in p15, p16, and p53 tumor suppressor genes are also frequently found in MF cases (45). In addition, SS may also have chromosomal amplification of the JUNB gene, which is a member of the activator protein-1 (AP-1) transcription factor complex involved in cell proliferation and T-helper 2 (Th2) cytokine expression by T cells (46). In early stages of MF, the tumor cells display a predominant Th1 cytokine profile, which shifts to a Th2 profile when the disease progresses to advanced MF/SS (47).

Using array-based comparative genomic hybridization, numerical chromosomal alterations most frequently observed in MF include gain of 7q21-36 and 1p36.2 and loss of 5q13 and 9q21, whereas SS frequently shows gain of 17q22-25 and 8q22-24 and loss of 17p13 and 10q24 (48). These findings suggest that MF and SS differ in molecular pathogenesis and thus therapeutic requirement may also be distinct (48). Microarray assay demonstrates the downregulation of miR-342 and upregulation of miR-199a* in SS samples (49). The collaboration of these two microRNAs may induce an antiapoptotic effect and thus play a role in the pathogenesis of SS (49). The salient features for laboratory diagnosis of MF/SS are summarized in Table 6.41.2.

Clinical Manifestations

MF/SS is usually seen in middle-aged adults with an average age of about 50 years (29). However, the disease can be seen in young adults (<20 years) or pediatric patients (50,51). The clinical course and pathologic findings are similar between early- and adult-onset MF, except that hypopigmentation is

TABLE 6.41.2

Salient Features of Laboratory Diagnosis of MF/SS

1. Skin biopsy shows characteristic histologic pattern.
2. Peripheral blood, bone marrow, and lymph node may show characteristic Sézary cells with cerebriform nuclei in SS.
3. Immunophenotype: Positive for CD2, CD3, CD4, and CD5
4. Important negative markers: CD7, CD8, CD1a, TdT
5. Immunogenotyping: TCR gene rearrangement in most cases
6. Electron microscopic identification of characteristic nuclei
7. Sézary cells are positive for TRAP, PAS, and b-glucuronidase.

MF, mycosis fungoides; PAS, periodic acid-Schiff; SS, Sézary syndrome; TCR, T-cell receptor; TdT, terminal deoxynucleotidyl transferase; TRAP, tartrate-resistant acid phosphatase.

more frequently seen in childhood MF (6,50,51). The male-to-female ratio is 2.2:1, and the black-to-white ratio is 2:1 (4). Clusters of cases within families have been reported, and an association with histocompatibility antigens, such as AW31, AW32, B8, BW35, and DR5, has been described.

The etiology of SS/MF is still not clear, but a retroviral cause has been suggested. The association of human T-cell leukemia virus type I with MF/SS is supported by evidence based on serology, molecular biology, and ultrastructural studies (1,4,52,53). However, other studies failed to substantiate these findings (1,4).

The clinical course of MF/SS is unusually indolent and is frequently preceded by a premalignant phase for several years. Many cases have an orderly progression from limited patches to generalized patches, plaques, and tumors. Patients may also have alopecia, palmoplantar hyperkeratosis, onychodystrophy (thickened nails), or ectropion (drooping eyelid) (1). Nodal and visceral involvement is usually seen in a later stage. Infection is still the major cause of death (54). Transformation into high-grade lymphomas and coexistence with second malignancies (colon and lung cancers) have been reported (19,55).

Lymphadenopathy has been found in 60% of patients at presentation (56). Histologic examination of these LNs usually reveals features of DL. However, when immunophenotypic, cytogenetic, or molecular techniques are used, neoplastic cells can be identified in up to 85% of these nodes (26,57).

The existence of leukemic phase depends on the stage of development: 12% in the plaque stage, 16% in the tumor stage, and 100% in patients with generalized erythroderma (58). When peripheral blood is involved, about 50% of patients have lymphadenopathy (58).

Bone-marrow involvement has been demonstrated in 21.7% of specimens in one report (59). An infiltrative

pattern in the marrow was related to an advanced stage with 100% peripheral blood involvement, 75% generalized erythroderma, and 65.2% nodal or visceral disease (59). In contrast, a nodular pattern in the marrow was seen in an earlier stage with 20% peripheral blood involvement and 20% nodal or visceral disease.

Visceral dissemination is most commonly seen in the liver, spleen, and lungs and is associated with short survival times (median, 25 months) (29). Epithelial surfaces, such as genitourinary tracts, are preferentially involved (29,60). Most patients with visceral involvement also have lymphadenopathy and circulatory Sézary cells (58).

The standard staging classification system for MF is the TUMB system first proposed by the National Cancer Institute (Table 6.41.3) (1). However, the original criterion

of using 5% abnormal lymphocytes in the peripheral blood as the cutoff point for blood involvement is being challenged. The current practice by many MF referral centers is to raise the threshold to the level of 20% abnormal lymphocytes or an absolute Sézary cell count of $\geq 1,000/\text{mL}$ to consider peripheral blood involvement (1,11). This stricter criterion is partly due to the fact that small numbers of Sézary-like cells can be seen in the peripheral blood in other conditions, such as benign inflammatory dermatoses, rheumatic disease, sepsis, and virus-induced lymphocytosis (12).

The treatment and the prognosis of MF/SS depend on the stage of the disease. For patients in the T1 and T2 stages, the treatment is limited to skin-targeted phototherapy (e.g., PUVA), topical application of nitrogen mustard or other chemicals, or radiation therapy (1,3,47,61). Multiagent chemotherapy is used only in systemic disease. Extracorporeal photopheresis has been reported to be an effective treatment for SS or erythrodermic MF (3).

The 5-year survival for stage I is 80% to 90%; stage II, 60% to 70%; stage III, 40% to 50%; and stage IV 25% to 35% (2). When transformation to large-cell lymphoma occurs, the mean survival time is 22 months because of the frequent presence of extracutaneous progression (20).

For the differential diagnosis among T-cell lymphomas and leukemias, the reader is referred to Case 20. The distinction between MF/SS and other CTCLs is sometimes difficult. However, other CTCLs differ from MF/SS in showing no protracted skin lesions; occasional spontaneous regression; the presence of mixed cellular components, including a large number of monocyte-macrophages, heterogeneous phenotype; and no leukemic phase (62).

TABLE 6.41.3

TUMB Staging System for CTCL

T (tumor)

- T₁: Cutaneous patches and/or plaques covering <10% of body surface
- T₂: Generalized patches and/or plaques covering >10% of body surface
- T₃: One or more cutaneous tumor nodules
- T₄: Generalized erythroderma

N (lymph node)

- N₀: LNs clinically normal and histologically negative for tumor cells
- N₁: LNs clinically enlarged but histologically negative
- N₂: LNs clinically normal but histologically positive
- N₃: LNs clinically enlarged and histologically positive

M (metastases, visceral organs)

- M₀: No visceral organ involvement
- M₁: Visceral involvement present

B (blood)

- B₀: Atypical circulating cells not present ($\leq 5\%$)
- B₁: Atypical circulating cells present ($> 5\%$)

Staging

- Ia: T₁, N₀, M₀, B₀
- Ib: T₂, N₀, M₀, B₀
- IIa: T₁₋₂, N₁, M₀, B₀
- IIb: T₃, N₀₋₁, M₀, B₀
- IIIa: T₄, N₀, M₀, B₀
- IIIb: T₄, N₁, M₀, B₀
- IVa: T₁₋₄, N₂₋₃, M₀, B₀
- IVb: T₁₋₄, N₀₋₃, M₁, B₁

LN, lymph node; TUMB, tumor, node, metastasis, blood.

REFERENCES

- Kim YH, Hoppe RT. Mycosis fungoides and the Sézary syndrome. *Semin Oncol*. 1999;26:276–289.
- Ralkiaer E, Jaffe ES. Mycosis fungoides and Sézary syndrome. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:216–220.
- Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. 2005;105:3768–3785.
- Diamandidou E, Cohen PR, Kurzrock R. Mycosis fungoides and Sezary syndrome. *Blood*. 1996;88:2385–2409.
- Glusac EJ. Criterion by criterion, mycosis fungoides. *Am J Dermatopathol*. 2003;25:264–269.
- Liu V, McKee PH. Cutaneous T-cell lymphoproliferative disorders: approach for the surgical pathologist: recent advances and clarification of confused issues. *Adv Anat Pathol*. 2002;9:79–100.
- Olsen E, Vonderheid E, Pimpinelli N, et al. Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization for Research and Treatment of Cancer (EORTC). *Blood*. 2007;110:1713–1722.
- Sun T, Susin M. *Differential Diagnosis of Lymphoid Disorders*. New York, NY: Igaku-Shoin; 1996:181–189.

9. Santucci M, Biggeri A, Feller AC, et al. Efficacy of histologic criteria for diagnosing early mycosis fungoides. An EORTC Cutaneous Lymphoma Study Group investigation. *Am J Surg Pathol.* 2000;24:40–50.
10. Shapino PE, Pinto FJ. The histologic spectrum of mycosis fungoides/Sézary syndrome (cutaneous T-cell lymphoma). *Am J Surg Pathol.* 1994;18:645–667.
11. Vonderheid EC, Bernengo MG, Burg G, et al. Update on erythrodermic cutaneous T-cell lymphoma: report of the International Society for Cutaneous Lymphoma. *J Am Acad Dermatol.* 2002;46:95–106.
12. Payne CM, Gasser L. Ultrastructural morphometry in the diagnosis of Sézary syndrome. *Arch Pathol Lab Med.* 1990;114:661–671.
13. Naeim F, Capostagno VJ, Johnson CE Jr, et al. Sézary syndrome: tartrate-resistant acid phosphatase in the neoplastic cells. *Am J Clin Pathol.* 1979;71:528–533.
14. Wood GS, Matthews MJ. Diagnosis of T-cell malignant lymphoproliferative disorders in the skin. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs.* 2nd ed. Philadelphia, PA: W. B. Saunders; 1995: 413–447.
15. Pereyo NG, Requena L, Galloway J, et al. Follicular mycosis fungoides. A clinicopathologic study. *J Am Acad Dermatol.* 1997;36:563–568.
16. Glusac EJ, Shapiro PE, McNiff JM. Cutaneous T-cell lymphoma: refinement in the application of controversial histologic criteria. *Dermatol Clin.* 1999;17:601–614.
17. Chen KR, Tanaka M, Miyakawa S. Granulomatous mycosis fungoides with small intestinal involvement and a fatal outcome. *Br J Dermatol.* 1998;138:522–525.
18. Vergier B, de Muret A, Beylor-Barry M, et al. Transformation of mycosis fungoides. Clinicopathological and prognostic features of 45 cases. *Blood.* 2000;95:2212–2218.
19. Salhany KE, Cousar JB, Greer JP, et al. Transformation of cutaneous T-cell lymphoma to large cell lymphoma: a clinicopathologic and immunologic study. *Am J Pathol.* 1988;132:265–277.
20. Barcos M. Mycosis fungoides. Diagnosis and pathogenesis. *Am J Clin Pathol.* 1993;99:452–458.
21. Wood GS. Benign and malignant cutaneous lymphoproliferative disorders including mycosis fungoides. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2002:1185–1233.
22. Weiss LM, Wood GS, Warnke RA. Immunophenotypic differences between dermatopathic lymphadenopathy and lymph node involvement in mycosis fungoides. *Am J Pathol.* 1985;120:179–185.
23. Haynes BF, Metzgar RS, Minna JD, et al. Phenotypic characterization of cutaneous T-cell lymphoma. *N Engl J Med.* 1981;304:1319–1323.
24. Knowles DM. Immunophenotypic and antigen receptor gene rearrangement analysis in T-cell neoplasia. *Am J Pathol.* 1989;134:761–785.
25. Bergman R, Faclieru D, Sahar D, et al. Immunophenotyping and T-cell receptor g gene rearrangement analysis as an adjunct to the histopathologic diagnosis of mycosis fungoides. *J Am Acad Dermatol.* 1998;134:761–785.
26. Michie SA, Abel EA, Hoppe RT, et al. Expression of T-cell receptor antigens in mycosis fungoides and inflammatory skin lesions. *J Invest Dermatol.* 1989;93:116–120.
27. Feng B, Jorgensen JL, Jones D, et al. Flow cytometric detection of peripheral blood involvement by mycosis fungoides and Sézary syndrome using T-cell receptor Vb chain antibodies and its application in blood staging. *Mod Pathol.* 2010;23:284–295.
28. Campbell JJ, Clark RA, Watanabe R, et al. Sézary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood.* 2010;116:767–771.
29. Stefanato CM, Tallini G, Crotty PL. Histologic and immunophenotypic features prior to transformation in patients with transformed cutaneous T-cell lymphoma. Is CD25 expression in skin biopsy samples predictive of large cell transformation in cutaneous T-cell lymphoma? *Am J Dermatopathol.* 1988;20:1–6.
30. Vermeer MH, Geelen FAMJ, Kummer JA, et al. Expression of cytotoxic proteins by neoplastic T cells in mycosis fungoides increases with progression from plaque stage to tumor stage disease. *Am J Pathol.* 1999;154:1203–1210.
31. Wain EM, Orchard GE, Mayou S, et al. Mycosis fungoides with a CD56+ immunophenotype. *J Am Acad Dermatol.* 2005;53:158–163.
32. Poszepczynska-Guigne E, Schiavon V, D'Incan M, et al. CD158k/KIR3DL2 is a new phenotypic marker of Sézary cells: relevance for the diagnosis and follow-up of Sézary syndrome. *J Invest Dermatol.* 2004;122:820–823.
33. Su MW, Dorocicz I, Dragnowska WH, et al. Aberrant expression of T-plastin in Sézary cells. *Cancer Res.* 2003;63:7122–7127.
34. Tang N, Gibson H, Germeroth T, et al. T-plastin (PL53) gene expression differentiates Sézary syndrome from mycosis fungoides and inflammatory skin diseases and can serve as a biomarker to monitor disease progression. *Br J Dermatol.* 2010;162:452–468.
35. Bernengo MG, Novelli M, Quaglino P, et al. The relevance of the CD4+ CD26– subset in the identification of circulating Sézary cells. *Br J Dermatol.* 2001;144:125–135.
36. Kuzel TM, Roenigk HH, Rosen ST. Mycosis fungoides and the Sézary syndrome. A review of pathogenesis, diagnosis and therapy. *J Clin Oncol.* 1991;9:1298–1313.
37. Girardi M, Heald PW, Wilson LD. The pathogenesis of mycosis fungoides. *N Engl J Med.* 2004;350:1978–1988.
38. Weiss LM, Hu E, Woods GS, et al. Clonal rearrangements of T-cell receptor genes in mycosis fungoides and dermatopathic lymphadenopathy. *N Engl J Med.* 1985;313:539–544.
39. Whittaker SJ, Smith NP, Jones RR, et al. Analysis of b, g, and d T-cell receptor genes in mycosis fungoides and Sézary syndrome. *Cancer.* 1991;68:1572–1582.
40. Russell-Jones R, Whittaker S. T-cell receptor gene analysis in the diagnosis of Sézary syndrome. *Dermatology.* 1999;199:8–14.
41. Bachelez H, Bioul L, Flageul B, et al. Detection of clonal T-cell receptor g gene rearrangements with the use of the polymerase chain reaction in cutaneous lesions of mycosis fungoides and Sézary syndrome. *Arch Dermatol.* 1995;131:1027–1031.
42. Dereure O, Balavoine M, Salles MT, et al. Correlation between clinical, histologic, blood, and skin polymerase chain reaction outcome in patients treated for mycosis fungoides. *J Invest Dermatol.* 2003;121:614–617.
43. Ferenczi K, Yawalkar N, Jones D, et al. Monitoring the decrease of circulating malignant T cells in cutaneous T-cell lymphoma during photopheresis and interferon therapy. *Arch Dermatol.* 2003;139:909–913.
44. Espinet B, Salido M, Pujol RM, et al. Genetic characterization of Sézary syndrome by conventional cytogenetics and cross-species color banding fluorescent in situ hybridization. *Haematologica.* 2004;89:165–173.
45. Smoller BR, Santucci M, Wood GS, et al. Histopathology and genetics of cutaneous T-cell lymphoma. *Hematol Oncol Clin North Am.* 2003;17:1277–1311.

46. Mao X, Orchard G, Lillington DM, et al. Amplification and overexpression of JUNB is associated with primary cutaneous T-cell lymphomas. *Blood*. 2003;101:1513–1519.
47. Querfeld C, Rosen ST, Guitart J, et al. The spectrum of cutaneous T-cell lymphomas: new insights into biology and therapy. *Curr Opin Hematol*. 2005;12:273–278.
48. van Doorn R, van Kester MS, Dijkman R, et al. Oncogenomic analysis of mycosis fungoides reveals major differences with Sézary syndrome. *Blood*. 2009;113:127–136.
49. Ballabio E, Mitchell T, van Kester MS, et al. MicroRNA expression in Sézary syndrome: identification, function, and diagnostic potential. *Blood*. 2010;116:1105–1113.
50. Quaglini P, Zaccagna A, Verrone A, et al. Mycosis fungoides in patients under 20 years of age. Report of 7 cases, review of the literature and study of the clinical course. *Dermatology*. 1999;199:8–14.
51. Garzon MC. Cutaneous T-cell lymphoma in children. *Semin Cutan Med Surg*. 1999;18:226–232.
52. Knobler RM, Rehle T, Grossman M, et al. Clinical evolution of cutaneous T-cell lymphoma in a patient with antibodies to human T-lymphotropic virus type I. *J Am Acad Dermatol*. 1987;17:903–909.
53. Wantzin GL, Thomsen K, Nissen NI, et al. Occurrence of human T-cell lymphotropic virus (type I) antibodies in cutaneous T-cell lymphoma. *J Am Acad Dermatol*. 1986;15:598–602.
54. Axelrod PI, Lorber B, Conderheid EC. Infections complicating mycosis fungoides and Sézary syndrome. *JAMA*. 1992;267:1354–1358.
55. Kantor AF, Curtis RE, Vonderhedi EC, et al. Risk of second malignancy after cutaneous T-cell lymphoma. *Cancer*. 1989;63:1612–1615.
56. Bunn PA, Lamberg SI. Report of the committee on staging and classification of cutaneous T-cell lymphomas. *Cancer Treat Rep*. 1979;63:1612–1615.
57. Bunn PA Jr, Huberman MS, Whang-Peng J, et al. Prospective staging evaluation of patients with cutaneous T-cell lymphomas. Demonstration of a high-frequency of extracutaneous dissemination. *Ann Intern Med*. 1980;93:223–230.
58. Sausville EA, Eddy JL, Malsuch RW, et al. Histopathology staging at initial diagnosis of mycosis fungoides and the Sézary syndrome. Definition of three distinctive prognostic groups. *Ann Intern Med*. 1988;109:372–382.
59. Carney DN, Bunn PA Jr. Manifestations of cutaneous T-cell lymphoma. *J Dermatol Surg Oncol*. 1980;6:369–377.
60. Weinstock MA, Horm JW. Mycosis fungoides in the United States—increasing incidence and descriptive epidemiology. *JAMA*. 1988;260:42–46.
61. Foss F. Mycosis fungoides and the Sézary syndrome. *Curr Opin Oncol*. 2004;16:421–428.
62. Maeda K, Takahashi M, Takatsuka N, et al. Cutaneous T-cell lymphoma differing from classical mycosis fungoides and Sézary syndrome. Clinical, histological and immunohistochemical studies of six cases. *J Dermatol*. 1990;17:226–234.

CASE 42

Subcutaneous Panniculitis-Like T-Cell Lymphoma

CASE HISTORY

A 58-year-old man presented with multiple subcutaneous nodule in the lower extremities for 1 month. Constitutional symptoms included fever, chills, malaise, and myalgias. The patient was treated with antibiotics and topical steroid preparation to no avail. His peripheral blood examination showed a normal leukocyte count with no anemia and thrombocytopenia. Blood chemistry profile was unremarkable. Physical examination of the lower limbs revealed multiple tender, erythematous, subcutaneous nodules with no ulceration. His liver and spleen were not palpable. No superficial lymphadenopathy was detected. A skin biopsy was taken (Figs. 6.42.1–6.42.4).

IMMUNOHISTOCHEMISTRY

The skin biopsy showed that the tumor cells were immunoreactive with CD3, CD8, and bF1, but were negative for CD4, CD20, CD79a, and CD56. Epstein-Barr virus encoded RNA in situ hybridization (EBER) was also negative.

MOLECULAR GENETICS

Cytogenetic study showed a normal male karyotype of 46 XY. T-cell receptor (TCR) gene rearrangement analysis revealed clonal rearrangement of TCR-g chain gene.

DISCUSSION

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a cutaneous cytotoxic T-cell lymphoma involving primarily the subcutaneous adipose tissue, simulating a panniculitis. Gonzalez et al. (1) first reported eight cases associated with hemophagocytic syndrome and an aggressive clinical course in 1991. Kumar et al. (2) confirmed that this is a distinct neoplasm of cytotoxic T-cell origin. Salhany et al. (3) further supported this finding and found that this tumor was composed of clonal ab T cell or gd T-cell population. Subsequently, this disease was recognized as a provisional entity in the Revised European-American Classification of Lymphoid Neoplasms (4) and in the European Organization for Research and Treatment of Cancer (EORTC) (5). Finally,

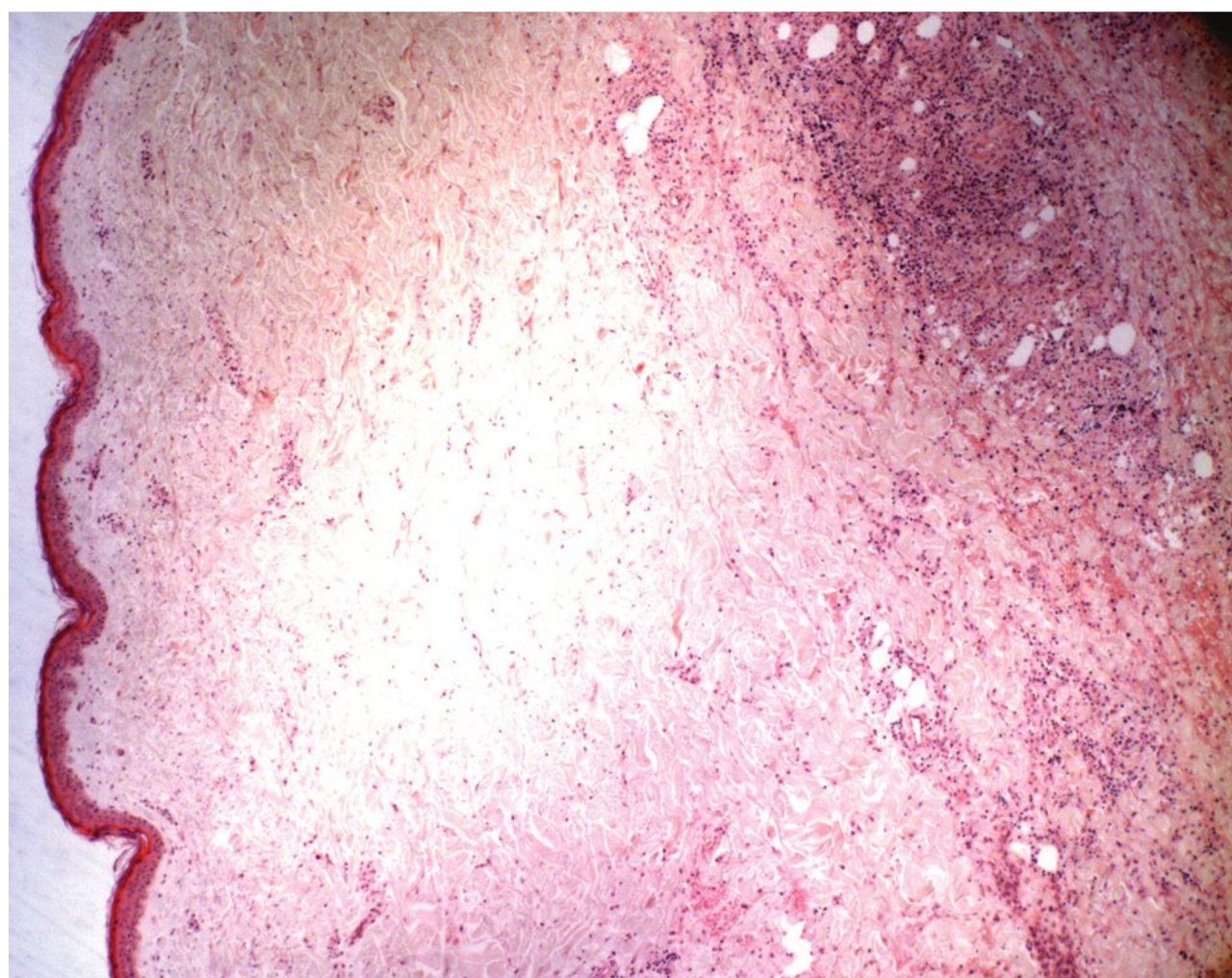


FIGURE 6.42.1 Skin biopsy shows cellular infiltration in the subcutaneous tissue. The dermis and epidermis are clear. H&E, $\times 5$.

SPTCL was defined as a distinct entity by the World Health Organization (WHO) classification in 2001 (6). Originally, SPTCL included both the ab T-cell phenotype and the gd T-cell phenotype; therefore, the clinical presentation and prognosis were heterogeneous in previous studies (7). In the light of recent studies, the WHO-EORTC classification redefined SPTCL to include only the a/b T-cell phenotype (8). The gd T-cell phenotype is now classified as primary cutaneous g/d T-cell lymphoma (PCTCL) (6).

MORPHOLOGY

The characteristic feature of SPTCL is that the tumor cell infiltration is predominantly in the fat lobules of the subcutaneous tissue, resembling a lobular panniculitis

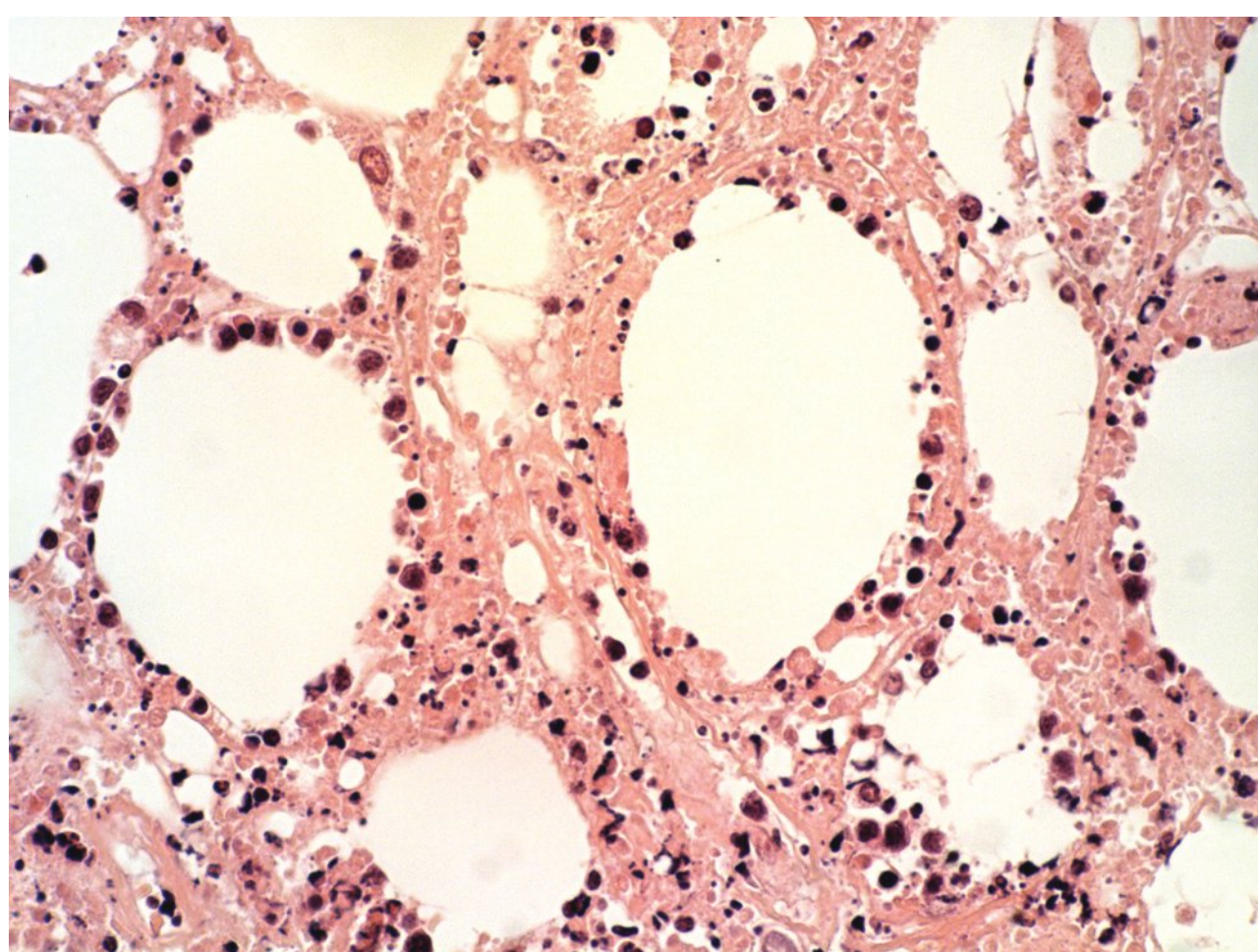


FIGURE 6.42.2 Skin biopsy shows rimming of individual adipocytes by lymphoma cells, forming a wreathlike pattern. H&E, $\times 40$.

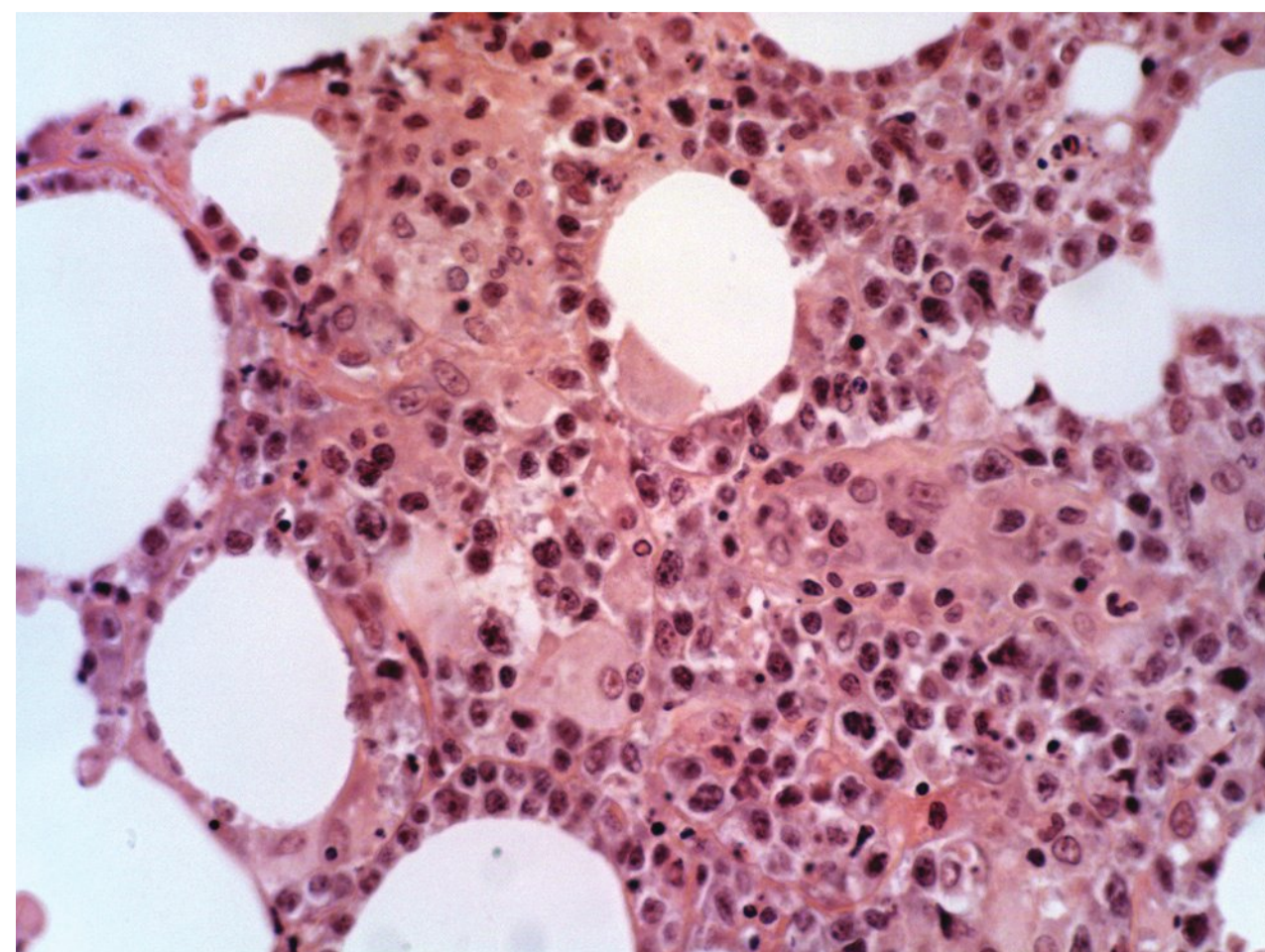


FIGURE 6.42.3 Skin biopsy shows hyperchromatic tumor cells intermixing with the lighter stained histiocytes. No plasma cells or neutrophils are present in the infiltrate. H&E, $\times 40$.

(6,9–11). Septal involvement is usually mild and represents secondary spillage of tumor cells from the lobules. The reticular dermis may show mild to moderate perivascular or periadnexal infiltration by the tumor cells. Neoplastic infiltration of the superficial dermis and epidermis is extremely rare.

The lymphoma cells are variable from small to medium to large size, but the cell size is usually constant in the same case. The neoplastic cells show irregular nuclei with a hyperchromatic chromatin pattern. Mitosis, apoptosis, and karyorrhexis are constant features. Fat necrosis is also common. The lymphoma cells are intermixed with small lymphocytes and histiocytes; the latter are often vacuolated due to ingestion of lipid substance. Neutrophils and eosinophils are generally absent unless there is focal necrosis. Plasma cells are also rarely seen except for the

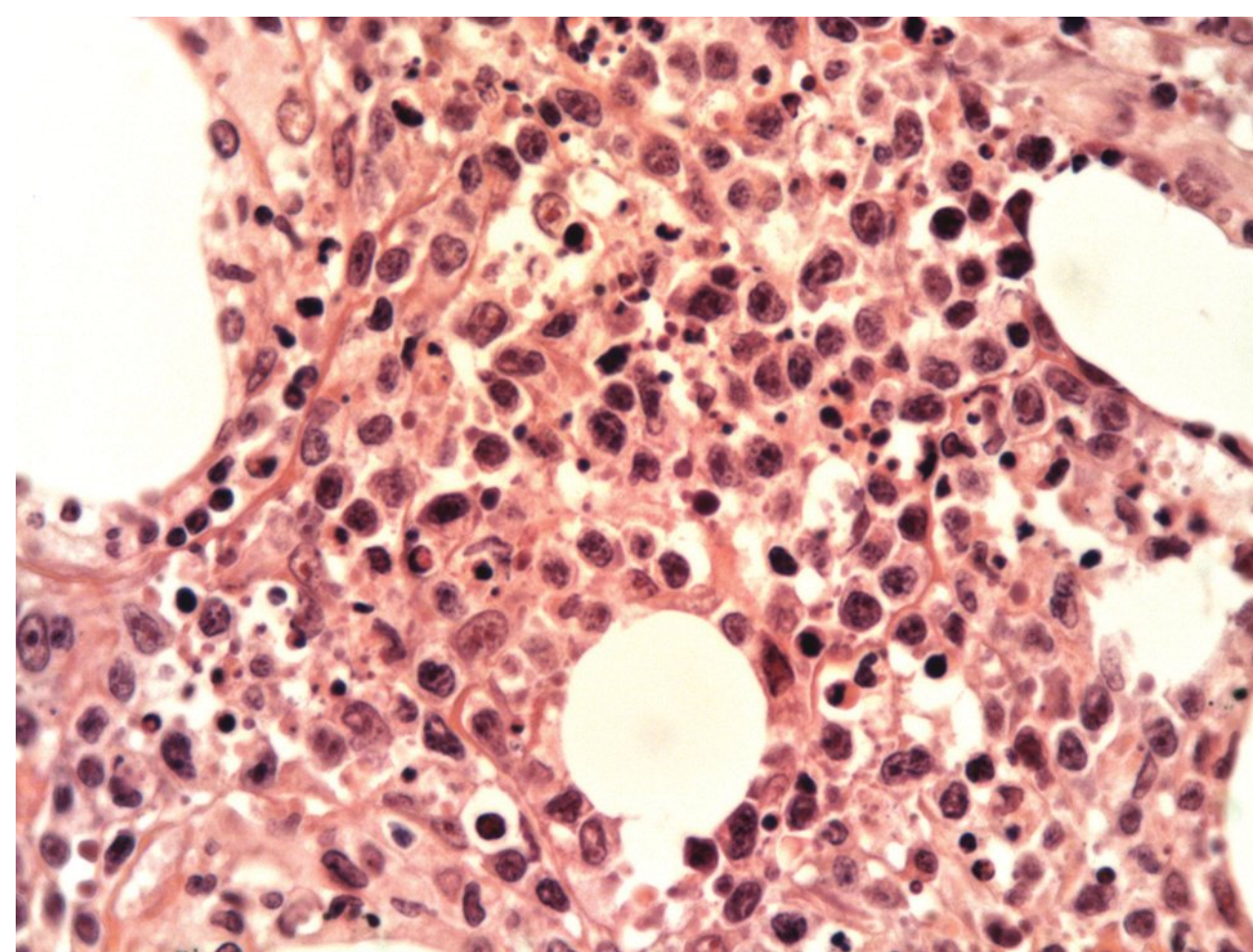


FIGURE 6.42.4 Higher magnification demonstrates many hyperchromatic, pleomorphic lymphoma cells and many apoptotic bodies and karyorrhexis. H&E, $\times 60$.

coexistence of lupus erythematosus (LE) (9). Granuloma with multinucleated giant cells is seen in occasional cases with fat necrosis (10). Cytophagocytosis of nuclear debris is common in a Chinese series, but erythrophagocytosis is rarely seen in cases with ab T-cell phenotype (10).

The most characteristic histologic pattern is the rimming of individual adipocytes by the lymphoma cells, forming a wreathlike pattern. However, this pattern is also seen in other cutaneous lymphoma as well as benign panniculitis and is not pathognomonic for SPTCL (12). Angioinvasion or angiodestruction is uncommon in Western patients, but relatively common in Chinese patients (9,10). For instance, 6 of 22 Chinese patients studied showed angiocentric infiltration and angioinvasion was seen in four cases. Cases with angioinvasion showed a statistically significant higher mortality rate than those without this feature (10).

In contrast to SPTCL, the PCTCL shows frequent involvement of the upper dermis and epidermis (9). In some cases, a marked lichenoid infiltrate with extensive necrosis of keratinocytes and ulceration is present. Angioinvasion and angiodestruction are commonly seen (9). Cytologic pleomorphism, apoptosis, and necrosis are also more prominent in cutaneous gd T-cell lymphoma than SPTCL (11). On the other hand, cutaneous natural killer-like T-cell lymphoma is also a cytotoxic T-cell lymphoma like SPTCL, but its morphologic features are similar to PCTCL with marked angiocentricity and angiodestruction, as well as a predominantly dermal infiltration (13). The presence of Epstein-Barr virus may help to distinguish it from the other two entities (9,13).

SPTCL should also be distinguished from LE. Histologically, LE shows epidermal involvement, vacuolar interface dermatitis, mucin depositions, presence of reactive germinal center, aggregation of B lymphocytes, and presence of considerable number of plasma cells (14–16). However, LE can be coexistent with SPTCL; therefore, the presence of these diagnostic features for LE does not exclude the presence of SPTCL (15,17). Other benign panniculitis usually show a heterogeneous mixed population, composed of lymphocytes, histiocytes, neutrophils, and plasma cells; while neutrophils and plasma cells are rare in SPTCL. In some cases, immunohistochemistry and gene rearrangement are needed to distinguish these two entities. For instance, benign panniculitis shows aggregates of B lymphocytes and mixed population of CD4- and CD8-positive T cells (11). T-cell gene rearrangement should show a germline pattern in benign lesions.

Immunophenotype

There are many cutaneous lymphomas that may show predominantly subcutaneous involvement and even the characteristic rimming pattern (6,12,18). These include PCTCL, extranodal NK/T-cell lymphoma, nasal type, primary cutaneous anaplastic large-cell lymphoma, mycosis fungoides, blastic plasmacytoid dendritic cell neoplasm, and cutaneous B-cell lymphomas. Under most circumstances, these tumors can be distinguished from SPTCL by clinicopathologic correlation, but from time to time, immunophenotyping is required for a definitive diagnosis.

The immunophenotype of SPTCL cells is CD3+, CD4-, CD8+, characteristic of cytotoxic T cells, which is also supported by the presence of the cytotoxic proteins, TIA-1, granzyme B, and perforin (6,9–11). The expression of bF1 in the tumor cells is indicative of the ab T-cell phenotype. On the other hand, the gd T-cell marker, TCRd1, is always negative. The naïve T-cell antigen, CD45RO, is consistently expressed, while the activated T-cell antigen, CD45RA, is absent (9). Loss of other T-cell markers, including CD2, CD5, and CD7, has been demonstrated in 10%, 50%, and 44% of cases, respectively, in a large study series (9). Mib-1 (Ki67) staining reveals high proliferation rates in most cases; only 6 of 53 cases studied had <25% positive cells (9).

The negative staining of CD56 and EBER on SPTCL cells helps to exclude extranodal NK/T cell lymphoma, nasal type (9,11). CD56 is positive in PCTCL and is considered a predictor of poor prognosis (19,20). However, EBER can be present in Asian patients with SPTCL due to the high incidence of Epstein-Barr virus infection in Asian countries (7,10). The absence of CD30 distinguishes SPTCL from primary cutaneous anaplastic large-cell lymphoma. Immunohistochemical stains with CD20 and CD79a usually demonstrate <10% scattered B cells in the lesion, which helps to exclude the diagnosis of cutaneous B-cell lymphoma (10). CD123 is a marker for plasmacytoid dendritic cells; therefore, it can be used to identify blastic plasmacytoid dendritic cell neoplasm (21), and it also stains some plasmacytoid dendritic cells in the lesion of LE (15). CD123 is negative in SPTCL.

Finally, it is important to distinguish PCTCL from SPTCL because these two entities show striking differences in their clinical course and prognosis. The gd T cells are usually CD3+, CD4-, CD8-, CD56+, bF1-, TCRd1+ (9,11). The distinction between these two entities is listed in Table 6.42.1.

Comparison between Flow Cytometry and Immunohistochemistry

Skin biopsy usually does not yield enough cells for flow cytometric analysis and most specimens received are already fixed in formalin because the lesion may look like benign panniculitis. Immunohistochemistry is the mainstay for the diagnosis, not only identifying all T-cell markers, but also TCR proteins, cytotoxic proteins, CD56, CD30, and EBER for differential diagnosis.

Molecular Genetics

TCR gene rearrangement is very important in identifying the T-cell clonality and distinguishing SPTCL from benign panniculitis. However, TCR gene rearrangement does not help to distinguish ab T cells from gd T cells, because TCR b, g, d chain genes are not cell lineage specific. For instance, in a study of 15 SPTCL patients, the frequency of rearranged TCRb, TCRg, and TCRd was 80%, 67%, and 13%, respectively (10).

Nonrandom karyotypic aberrations have not been reported in SPTCL cases (6). It is difficult to obtain molecular genetic data on SPTCL cases, because the malignant cells in the subcutaneous tissue are surrounded by fat cells, reactive T cells, and other normal cells in the skin,

TABLE 6.42.1		
Distinguishing Features Between SPTCL and PCTCL		
	SPTCL	PCTCL
Clinical course	Indolent	Aggressive
Hemophagocytic syndrome	Seldom seen	Frequent
Histologic location	Subcutaneous tissue	Subcutaneous tissue, dermis, and epidermis
TCR	bF1+, TCRd1–	bF1–, TCRd1+
T-cell phenotype	CD3+, CD4–, CD8+	CD3+, CD4–, CD8–
CD56	Negative	Commonly expressed
5-yr overall survival, %	82	11

PCTCL, primary cutaneous $\gamma\delta$ T-cell lymphoma; SPTCL, subcutaneous panniculitis-like T-cell lymphoma.

and the lymphoma cells seldom circulate in the peripheral blood (22). With microdissection and DNA amplification techniques, Hahtola et al. (22) obtained enough pure tumor DNA for comparative genomic hybridization. This study found large numbers of DNA copy number changes, which included losses in chromosomes 1p, 2p, 2q, 5p, 7p, 9q, 10q, 11q, 12q, 16, 17p, 19, 20, 22, and gains in chromosomes 2q, 4q, 5q, 6q, and 13q. This group also identified the deletion of a tumor suppressor gene, NAV3, at 12q21 by fluorescence in situ hybridization and loss of heterozygosity analysis (22).

Deletion and translocation of NAV3 have also been found in other primary cutaneous T-cell lymphomas (23). The aberrations in chromosome regions 10q, 17p, and 19 are also shared by SPTCL with other cutaneous T-cell neoplasms, namely, mycosis fungoides and Szary syndrome. However, 5q and 13q gains are characteristic of SPTCL (22). The authors concluded that molecular genetic studies confirm SPTCL as a distinct entity in the category of cutaneous T-cell lymphoma (22).

The current case showed lesions exclusively in the subcutis with hyperchromatic atypical lymphoid cells infiltrating the adipose tissue. Rimming of adipocytes by lymphoma cells was also demonstrated. Furthermore, immunohistochemical stains demonstrated CD3, CD8, and bF1 staining of the tumor cells, but no staining for CD4, CD20, CD79a, and CD56. EBER was also negative. Clonal TCR γ chain gene rearrangement was detected. Therefore, this case is consistent with SPTCL morphologically and immunophenotypically. The salient features for laboratory diagnosis of SPTCL are summarized in Table 6.42.2.

Clinical Manifestation

SPTCL is a rare disease, accounting for <1% of all non-Hodgkin lymphomas. It is usually seen in a younger population with a median age of 35 years and 20% of patients are under the age of 20 (6). The major clinical presentation is the presence of multiple subcutaneous nodules or deep-seated plaques, most frequently seen on the extremities and trunk (6,9–11). Systemic symptoms, such as fever, chills, night

sweats, weight loss, and myalgia, are seen in up to 50% of patients. Cytopenia and abnormal liver function tests are common laboratory findings. Hemophagocytic syndrome is identified in 15% to 20% of cases and is usually associated with poor prognosis (7,9,11). However, in a study of 22 Asian patients with SPTCL, none of them showed this syndrome (10). Lymphadenopathy and hepatosplenomegaly are rarely seen in SPTCL patients, and these organs do not have lymphomatous involvement. (6,9,10).

One interesting clinical observation is the association of autoimmune disorders, particularly LE, in some SPTCL patients (6,9,15). While some authors consider LE and SPTCL two distinct entities (14), others consider them a spectrum of the same disease as they share many overlapping clinicopathologic features (17). The intriguing question is whether patients with LE are at higher risk of developing SPTCL or patients with SPTCL is susceptible to developing autoimmune antibodies and even clinical

TABLE 6.42.2	
Salient Features for Laboratory Diagnosis of Subcutaneous Panniculitis-like T-cell Lymphoma	
1. Morphology:	Lesion is confined to subcutaneous tissue with rimming of individual adipocytes by lymphoma cells, forming a wreathlike pattern.
2. Immunophenotype:	CD3+, CD4–, CD8+, bF1+, TCRd1–
3. Important negative markers for differential diagnosis:	CD20, CD30, CD56, CD79a, CD123, and EBER
4. Gene rearrangement:	Either TCRb or TCRg chain gene rearrangement is demonstrated.
5. Molecular genetics:	No nonrandom karyotypic abnormality. Deletion of tumor suppressor gene NAV3 at 12q21

autoimmune phenomenon, such as LE. (15). To this aspect, it is interesting to note that autoimmune phenomenon disappeared after successful treatment of four SPTCL patients in one study series (22).

With the new restricted diagnostic criteria for SPTCL, most patients have an indolent clinical course with a favorable prognosis and respond well to conservative therapy. Therefore, some authors question whether aggressive therapy, such as CHOP, is necessary for the treatment of this disease (9).

REFERENCES

- Gonzalez CL, Medeiros LJ, Braziel RM, et al. T-cell lymphoma involving subcutaneous tissue: a clinicopathologic entity commonly associated with hemophagocytic syndrome. *Am J Surg Pathol*. 1991;15:17–27.
- Kumar MS, Krenacs L, Medeiros J, et al. Subcutaneous panniculitic T-cell lymphoma is a tumor of cytotoxic T lymphocytes. *Hum Pathol*. 1998;29:397–403.
- Salhany KE, Macon WR, Choi JK, et al. Subcutaneous panniculitis-like T-cell lymphoma: clinicopathologic, immunophenotypic, and genotypic analysis of alpha/beta and gamma/delta subtypes. *Am J Surg Pathol*. 1998;22:881–893.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
- Willemze R, Keri H, Sterry W, et al. EORTC classification for primary cutaneous lymphomas: a proposal from the Cutaneous Lymphoma Study Group of The European Organization for Research and Treatment of Cancer. *Blood*. 1997;90:354–371.
- Jaffe ES, Gaulard P, Ralfkiaer E, et al. Subcutaneous panniculitis-like T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:204–205.
- Go RS, Wester SM. Immunophenotypic and molecular features, clinical outcomes, treatments, and prognostic factors associated with subcutaneous panniculitis-like T-cell lymphoma. *Cancer*. 2004;101:1404–1413.
- Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphomas. *Blood*. 2005;105:3768–3785.
- Willemze R, Jansen PM, Cerroni L, et al. Subcutaneous panniculitis-like T-cell lymphoma: definition, classification, and prognostic factors: an EORTC Cutaneous Lymphoma Group Study of 83 cases. *Blood*. 2008;111:838–845.
- Kong YY, Dai B, Kong JC, et al. Subcutaneous panniculitis-like T-cell lymphoma: a clinicopathologic, immunophenotypic, and molecular study of 22 Asian cases according to WHO-EORTC Classification. *Am J Surg Pathol*. 2008;32:1495–1902.
- Parveen Z, Thompson K. Subcutaneous panniculitis-like T-cell lymphoma: redefinition of diagnostic criteria in the recent World Health Organization-European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas. *Arch Pathol Lab Med*. 2009;133:303–308.
- Lozzi GP, Massone C, Citarella L, et al. Rimming of adipocytes by neoplastic lymphocytes: A histopathologic feature not restricted to subcutaneous T-cell lymphoma. *Am J Dermatopathol*. 2006;28:9–12.
- Yamashita Y, Tsuzuki T, Nakayama A, et al. A case of natural killer/T-cell lymphoma of the subcutis resembling subcutaneous panniculitis-like T cell lymphoma. *Pathol Int*. 1999;49:241–246.
- Massone C, Kodama K, Salmhofer W, et al. Lupus erythematosus panniculitis (lupus profundus): clinical, histopathological, and molecular analysis of nine cases. *J Cutan Pathol*. 2005;32:396–404.
- Pincus LB, LeBoit PE, McCalmont TH, et al. Subcutaneous panniculitis-like T-cell lymphoma with overlapping clinicopathologic features of lupus erythematosus: Coexistence of 2 entities? *Am J Dermatopathol*. 2009;31:520–526.
- Cassis TB, Fearneyhough PK, Callen JP, et al. Subcutaneous panniculitis-like T-cell lymphoma with vacuolar interface dermatitis resembling lupus erythematosus panniculitis. *J Am Acad Dermatol*. 2004;50:465–469.
- Magro CM, Crowson AN, Kovatich AJ, et al. Lupus profundus, indeterminate lymphocytic lobular panniculitis and subcutaneous T-cell lymphoma: a spectrum of subcuticular T-cell lymphoid dyscrasia. *J Cutan Pathol*. 2001;28:235–247.
- Massone C, Lozzi GP, Egberts F, et al. The protean spectrum of non-Hodgkin lymphomas with prominent involvement of subcutaneous fat. *J Cutan Pathol*. 2006;33:418–425.
- Takeshita M, Imayama S, Oshiro Y, et al. Clinicopathologic analysis of 22 cases of subcutaneous panniculitis-like CD56– or CD56+ lymphoma and review of 44 other reported cases. *Am J Clin Pathol*. 2004;121:408–416.
- Kao GF, Resh B, McMahon C, et al. Fatal subcutaneous panniculitis-like T-cell lymphoma g/d subtype (cutaneous g/d T-cell lymphoma): Report of a case and review of the literature. *Am J Dermatopathol*. 2008;30:593–599.
- Facchetti F, Jones DM, Petrella T. Blastic plasmacytoid dendritic cell neoplasm. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:145–147.
- Hahtola S, Burghart E, Jeskanen L, et al. Clinicopathological characterization and genomic aberrations in subcutaneous panniculitis-like T-cell lymphoma. *J Invest Dermatol*. 2008;128:2304–2309.
- Karenko L, Hahtola S, Palvinen S, et al. Primary cutaneous T-cell lymphomas show a deletion or translocation affecting NAV3, the human UNC-53 homologue. *Cancer Res*. 2005;65:8101–8110.

CASE 43

Peripheral T-Cell Lymphoma, Not Otherwise Specified

CASE HISTORY

A 65-year-old woman presented with low-grade fever, night sweats, and weight loss for several months. Physical examination by her private care physician revealed splenomegaly and multiple lymphadenopathies, involving cervical, retroperitoneal, and mediastinal lymph nodes. No hepatomegaly was found. Peripheral blood examination was essentially normal with a total leukocyte count of 4,600/mL, hematocrit 45.4%, and platelets 156,000/mL. She was admitted to the hospital for further studies. A cervical lymph node biopsy was performed and was diagnosed as peripheral T-cell lymphoma (PTCL). A bone marrow biopsy, however, showed no lymphoma involvement.

She was treated with chemotherapy and did not respond to the treatment at first. After switching to another regimen, she showed symptomatic improvement as well as shrinkage of lymph nodes in the mediastinum and retroperitoneum. However, she suffered neutropenic fever and peripheral neuropathy in the lower extremities.

Two years later, multiple skin nodules were found on her left forearm and both shoulders. Skin biopsy showed B-cell lymphoma, and subsequent skin biopsy 1 year later revealed amyloid deposits. Nevertheless, a mucosal biopsy did not detect amyloidosis, and echocardiogram showed no amyloid cardiac involvement. Serum protein electrophoresis and immunoglobulin quantitation revealed no evidence of myeloma.

She continued to receive chemotherapy as well as local radiation of the skin lesions. The patient was followed in the hematology/oncology clinic for periodic evaluation of her skin lesions, adenopathy, hepatosplenomegaly, complete blood cell counts, and lactate dehydrogenase levels.

FLOW CYTOMETRIC FINDINGS

Lymph node biopsy: T-cell markers: CD2 88%, CD3 82%, CD3/CD4 70%, CD3/CD8 6%, CD5 80%, CD7 51%; B-cell markers: CD19 14%, CD20 12%, k 8%, l 4% (Fig. 6.43.1).

IMMUNOHISTOCHEMICAL STAINS

The lymph node biopsy showed positive stains for CD3, CD4, CD5, CD43, and CD45, but negative stains for CD8, CD20, and Alk1 on the tumor cells.

DISCUSSION

PTCL is composed of a heterogeneous group of T-cell tumors with different clinical and morphologic features.

The Working Formulation (1) and the Kiel scheme (2) classify PTCL mainly on the basis of morphology. The Revised European-American classification of lymphoid neoplasms and the World Health Organization (WHO) define PTCL by comprehensive clinicopathological features (3–5). Clinically, PTCL can be divided into three groups: nodal, extranodal, and leukemic/disseminated (5,6), and each clinical subtype contains several well-defined entities (Table 6.43.1). T-cell tumors that cannot be classified into one of those entities is designated as peripheral T-cell lymphoma, unspecified (PTCL-U) or PTCL, not otherwise specified. PTCL accounts for 7% to 10% of all non-Hodgkin lymphomas (7). However, the frequency of PTCL varies greatly in different geographic regions. One study of PTCL (excluding anaplastic large-cell lymphoma) showed that the frequency varied from 1.5% in Canada to 18.3% in Hong Kong (8). Among the PTCLs, the most common entities are PTCL-U, angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma, and cutaneous T-cell lymphoma (8). These four entities account for approximately 80% of all PTCLs in the United Kingdom (8).

Morphology

PTCL-U is usually a nodal lymphoma with extensive infiltration of the lymph node and effacement of the normal architecture (4,9,10). There is a great variation of cytologic features. Most cases show a mixture of large and small tumor cells, but predominantly large cells or predominantly small cells can also be found in some cases (Table 6.43.2). The nuclear configuration is usually irregular, and the cytoplasm is frequently transparent or lightly stained (clear cells) (Fig. 6.43.2). Although clear cells can be seen in B-cell lymphomas, the presence of clear cells should raise the suspicion of a T-cell lymphoma. The chromatin pattern in large tumor cells is often vesicular or dispersed with or without the presence of nucleoli. The small tumor cells usually show a clumped chromatin pattern without nucleoli. Even in the small-cell variant, large cells, multinucleated giant cells, or Reed-Sternberg-like cells may be present (Fig. 6.43.3). In addition, eosinophils, plasma cells, and epithelioid histiocytes can be seen in the background; these reactive cell components are attributed by the cytokines released from the lymphoma cells (11).

Cases that show Reed-Sternberg-like cells and an inflammatory background are difficult to distinguish from Hodgkin lymphoma. Careful inspection of the lymphoid population and the presence of other Hodgkin tumor cells may help to differentiate these two entities, but immunophenotyping or genotyping is frequently required to make the distinction. The T-cell-/histiocyte-rich B-cell lymphoma may also show a mixture of small lymphoid cells and histiocytes mimicking PTCL-U, but the small number of neoplastic B cells can be identified with immunohistochemical staining.

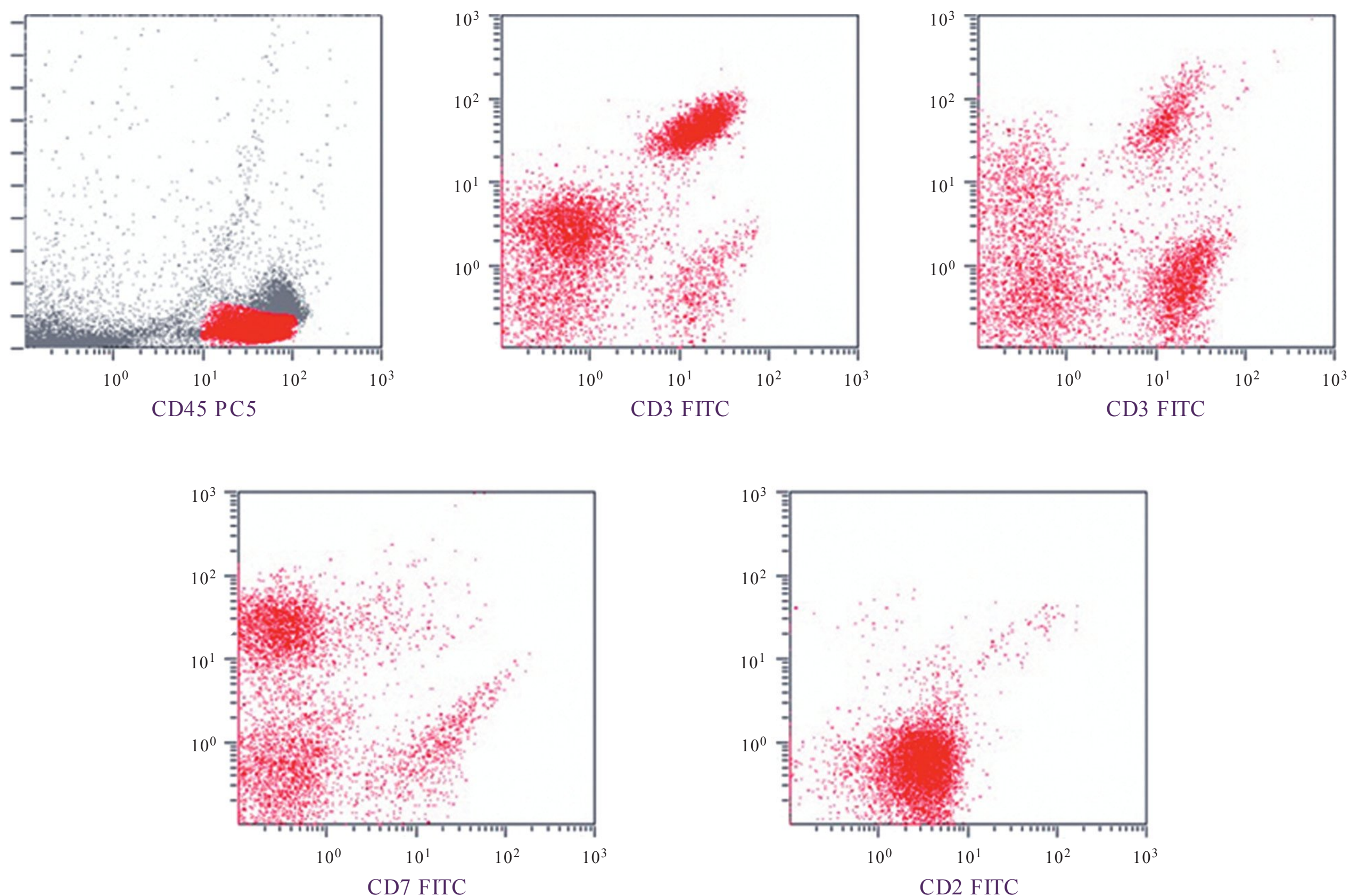


FIGURE 6.43.1 Flow cytometric analysis of the lymph node shows predominance of CD3/CD4 over CD3/CD8 staining. CD2 and CD5 are positive, but CD7 shows a lower percentage than other T-cell markers. SS, side scatter; PC5, phycoerythrin-cyanin 5; RD1, rhodamine; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

Although it is not a common feature, high endothelial venules and even arborizing blood vessels can be seen in some cases of PTCL-U (Fig. 6.43.4). These cases have to be distinguished from AITL. The latter may show a prominent angiocentric pattern in the paracortical region with the presence of a follicular dendritic meshwork (Fig. 6.43.5).

Bone marrow involvement is present in one third of PTCL-U cases (12). The histologic pattern can be interstitial, intrasinusoidal, diffuse, or nodular, and is thus indistinguishable from other lymphomas. Three specific morphologic variants are included in the PTCL-U category, but no specific clinical features are associated with them (5,13).

Lymphoepithelioid Variant (Lennert Lymphoma)
Tumor cells in this variant are usually composed of small lymphoid cells with slightly irregular nuclei, clumped chromatin pattern, and absence of nucleoli (10,14–16). There are also small numbers of medium-sized or large cells present. The infiltration pattern can be diffuse or interfollicular. The characteristic feature is an exuberant proliferation of epithelioid histiocytes (Figs. 6.43.6 and 6.43.7), presumably a response to lymphokines released by the tumor cells (10). The epithelioid histiocytes, in turn, may secrete interleukin-6 to stimulate the proliferation of the tumor cells (16).

The frequent presence of eosinophils, plasma cells, and Reed-Sternberg-like cells in this entity makes it difficult to distinguish from Hodgkin lymphoma. As mentioned before,

immunophenotyping or genotyping is frequently required to make the distinction.

Lymphoepithelioid lymphoma (LEL) is somewhat similar to T-cell/histiocyte-rich B-cell lymphoma in that, as the disease progresses, the tumor cells become more prominent and the epithelial component is gradually diminished (10,14). LEL can also transform into AITL, T-zone lymphoma, or large-cell lymphoma (10,15,17).

T-Zone Variant

In this variant, the tumor cell infiltrate is concentrated in the interfollicular area (T-cell zone) (4,15,18,18,19). The lymphoid follicles are well preserved or even hyperplastic. The tumor cells are usually of small- to medium-size with slightly irregular nuclear configuration. Clear cells can be seen, and high endothelial venules may be increased in some cases. Those cases should be distinguished from AITL. In the background, epithelioid histiocytes, eosinophils, and plasma cells are frequently present, mimicking LEL. When Reed-Sternberg-like cells are present, this tumor should be distinguished from Hodgkin lymphoma.

Follicular Variant

This recently recognized entity is included in the 2008 WHO Classification as one of the three variants (5). Histologically, several different patterns have been reported. It can be a true follicular pattern mimicking follicular lymphoma (20);

TABLE 6.43.1	
Classification of PTCL	
Clinical presentation	Lymphoma subtype
Nodal	Angioimmunoblastic T-cell lymphoma
	Anaplastic large-cell lymphoma
	Peripheral T-cell lymphoma, not otherwise specified
Extranodal	Mycosis fungoides
	Primary cutaneous anaplastic large-cell lymphoma
	Hepatosplenic T-cell lymphoma
	Subcutaneous panniculitis-like T-cell lymphoma
	Enteropathy-type T-cell lymphoma
	Extranodal NK/T-cell lymphoma, nasal type
	EBV-positive T-cell lymphoproliferative disorder of childhood
	Primary cutaneous T-cell lymphoma, rare types
Leukemic/disseminated	T-cell prolymphocytic leukemia
	T-cell large granular lymphocytic leukemia
	Adult T-cell leukemia/lymphoma
	Aggressive NK-cell leukemia
	Chronic lymphoproliferative disorder of NK cells

NK, natural killer.

a nodular paracortical or perifollicular growth pattern, similar to marginal zone lymphoma (21–23); and cellular aggregates within expanded mantle zone (24). The tumor cells are variable in size. Some cases show medium-sized cells with abundant clear cytoplasm, while others reveal morphology resembling centrocytes and centroblasts. A definitive diagnosis depends on the demonstration of a follicular helper T-cell phenotype (BCL-6+, CXCL13+, PD-1+), mimicking AITL. However, the absence of prominent high endothelial venules and of enlarged follicular dendritic cell meshworks in PTCL-U distinguishes it from AITL (5).

Immunophenotype

The characteristic immunophenotype of PTCL-U is the absence of thymic T-cell markers (CD1a and terminal deoxynucleotidyl transferase [TdT]) (14,25) and the presence of peripheral pan-T-cell markers (CD2, CD3, CD5, and/or CD7) (14,26). However, selective loss of one or more pan-T-cell markers is a common finding, and this aberrant immunophenotype helps to identify T-cell neoplasm (27). The only exception is LEL, which seldom shows this phenomenon (26). Unlike B-cell lymphomas, there are no monoclonal

TABLE 6.43.2	
Characteristic Morphologic Features of PTCL-U	
Histologic pattern	Diffuse pattern in lymphoepithelioid variant; interfollicular infiltration in T-zone lymphoma, and follicular or perifollicular pattern in follicular variant. High endothelial venules may be present.
Cytology	Mixed small and large cells, predominantly small cells or predominantly large cells Clear cytoplasm is characteristic but not always present. Multinucleated giant cells and Reed-Sternberg-like cells may be present.
Special features	Diffuse tumor cell infiltration with epithelioid histiocytes, eosinophils, and/or plasma cells in the background is characteristic for lymphoepithelioid lymphoma The presence of clear cells and high endothelial venules is characteristic.

PTCL-U, peripheral T-cell lymphoma, not otherwise specified.

markers for T-cell tumors. PTCL-U may be presented with a predominantly CD4 (helper/inducer) or CD8 (suppressor/cytotoxic) phenotype. For instance, LEL is of CD8+ phenotype and T-zone lymphoma expresses predominantly CD4 marker (Fig. 6.43.8). However, the predominance of CD4 or CD8 cells is not a reliable indicator of monoclonality because it may also be demonstrated in reactive

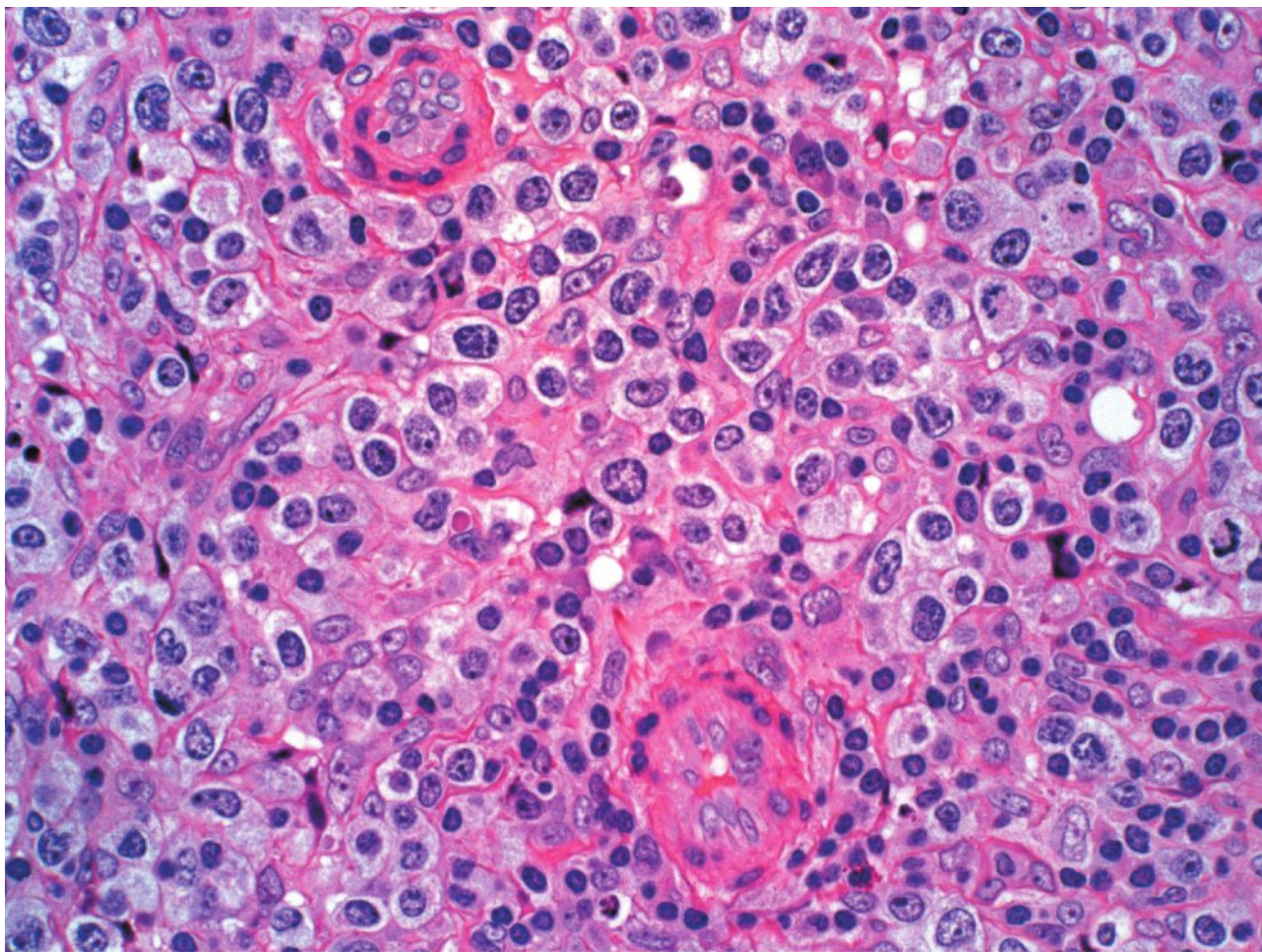


FIGURE 6.43.2 Lymph node biopsy of a PTCL shows tumor cells with clear cytoplasm and pleomorphic nuclei. There are two high endothelial venules present in this field. Hematoxylin and eosin, 40× magnification.

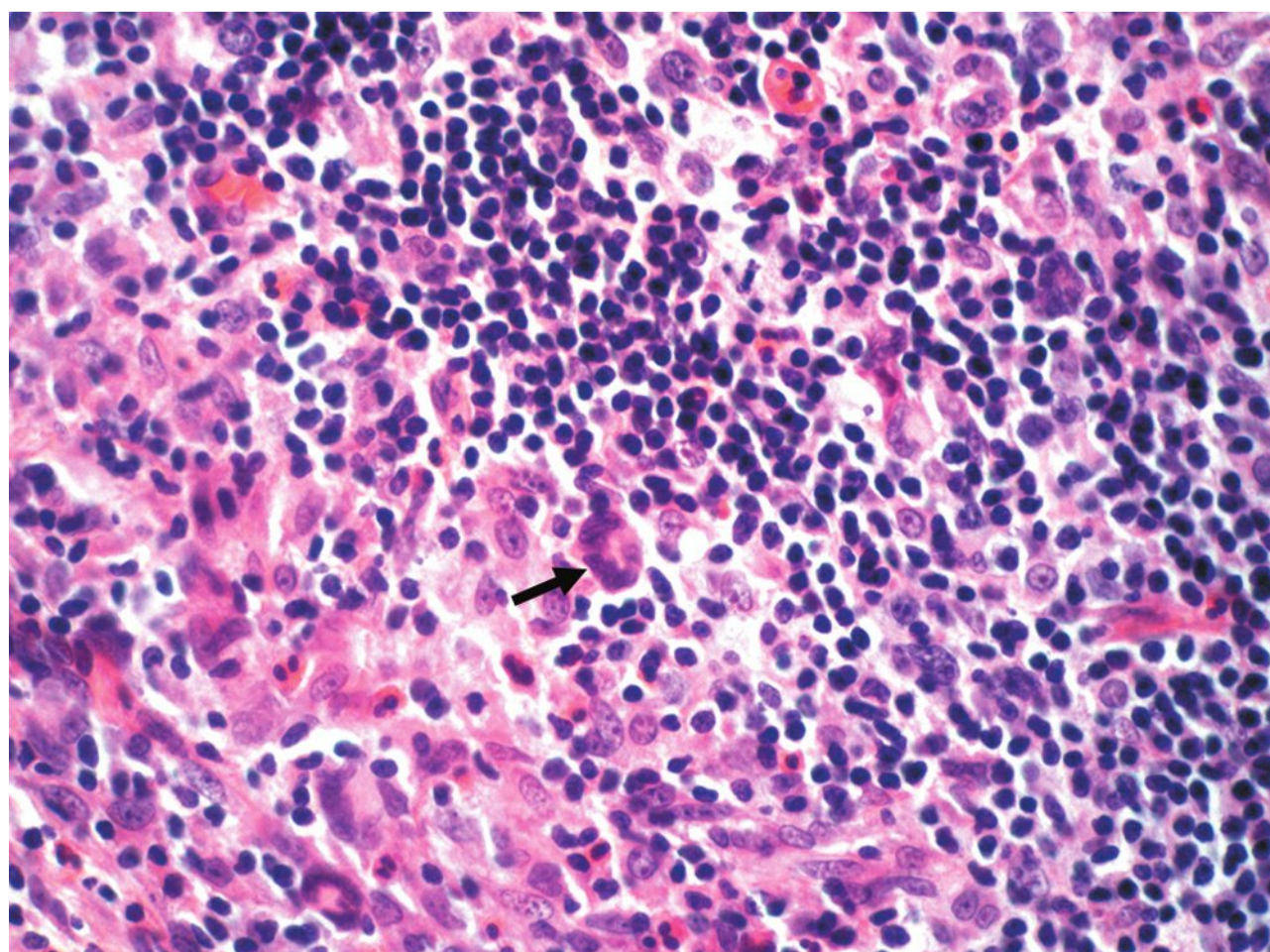


FIGURE 6.43.3 Lymph node biopsy of lymphoepithelioid lymphoma shows a few multinucleated giant cells (arrow) in this field. Hematoxylin and eosin, 40× magnification.

lymphoproliferation. In fact, LEL may show predominant CD4 staining and yet the atypical tumor cells are marked for CD8 (28). In the early literature, LEL was considered a CD4+ helper T-cell lymphoma based on immunostaining of frozen sections. It is now realized that the CD8+ tumor cells may be embedded in clusters of CD4+ epithelioid histiocytes with resultant misleading interpretation (28). Nevertheless, when both CD4 and CD8 are absent or when there is coexpression of both CD4 and CD8 in the same population, T-cell lymphoma should be suspected.

The follicular variant is characterized by an immunophenotype of CD3+, CD4+, CD8–, BCL-6+, CD10± (20,23,24,29). A recent study found that CXCL13 was identified in 96% of PTCL-U (excluding AITL) cases, probably containing mostly the follicular variant (29). Programmed death-1 (PD-1) expression in this group of lymphomas was

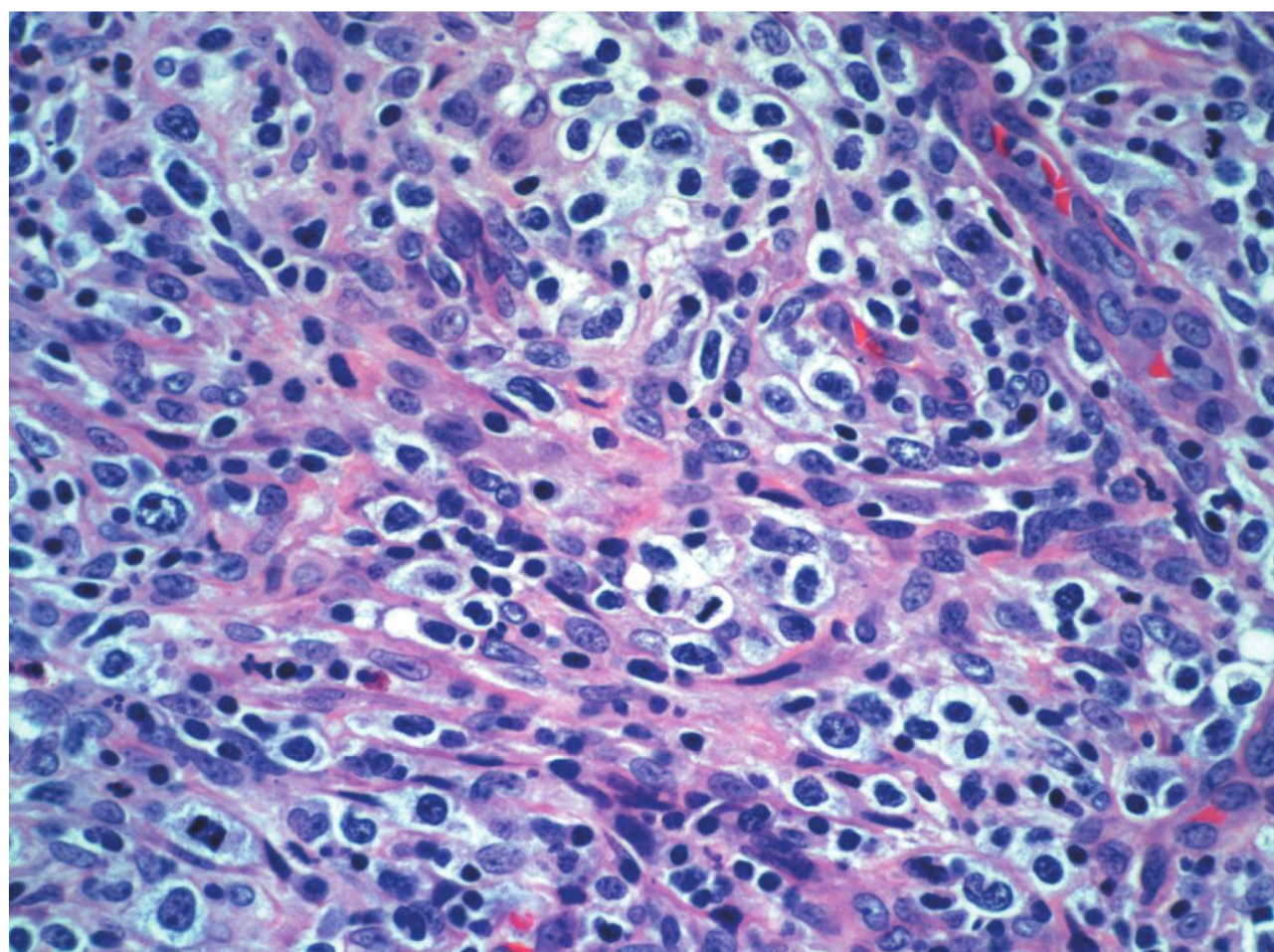


FIGURE 6.43.4 Lymph node biopsy of a PTCL shows arborizing blood vessels. Hematoxylin and eosin, 40× magnification.

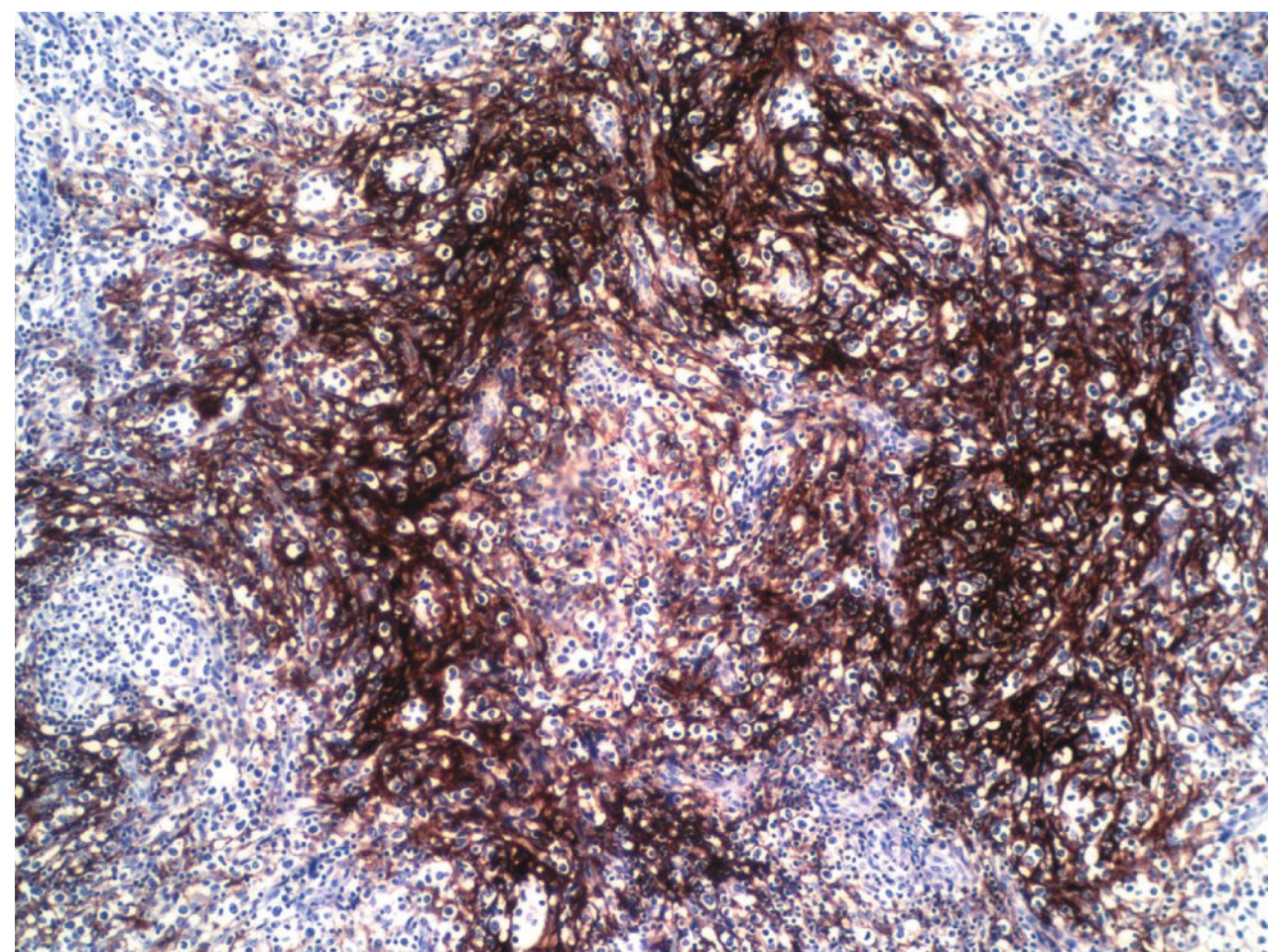


FIGURE 6.43.5 Lymph node biopsy of angioimmunoblastic T-cell lymphoma shows a follicular dendritic meshwork with CD21 stain. Immunoperoxidase, 10× magnification.

also demonstrated by tissue arrays using a monoclonal antibody, NAT-105 (29).

Double staining with Ki-67/CD4 and Ki-67/CD8 may also help to distinguish neoplastic from normal T-helper cells (16). The tumor cells are Ki-67 positive, and the normal T-cells are negative. When CD4 and CD8 were applied without Ki-67, a large number of cases showed almost equal numbers of CD4- and CD8-positive cells in one study (15). However, when frozen sections were double stained with Ki-67/CD4 and Ki-67/CD8, all cases showed a Ki-67-/CD4-predominant phenotype.

In immunohistochemical stains, CD2, CD3, CD4, CD5, CD7, CD43, and/or CD45RO can be detected in the tumor cells (26). Selective loss of one or more pan-T-cell markers is frequently found by immunohistochemistry just the same as in flow cytometry. It should be emphasized that the presence

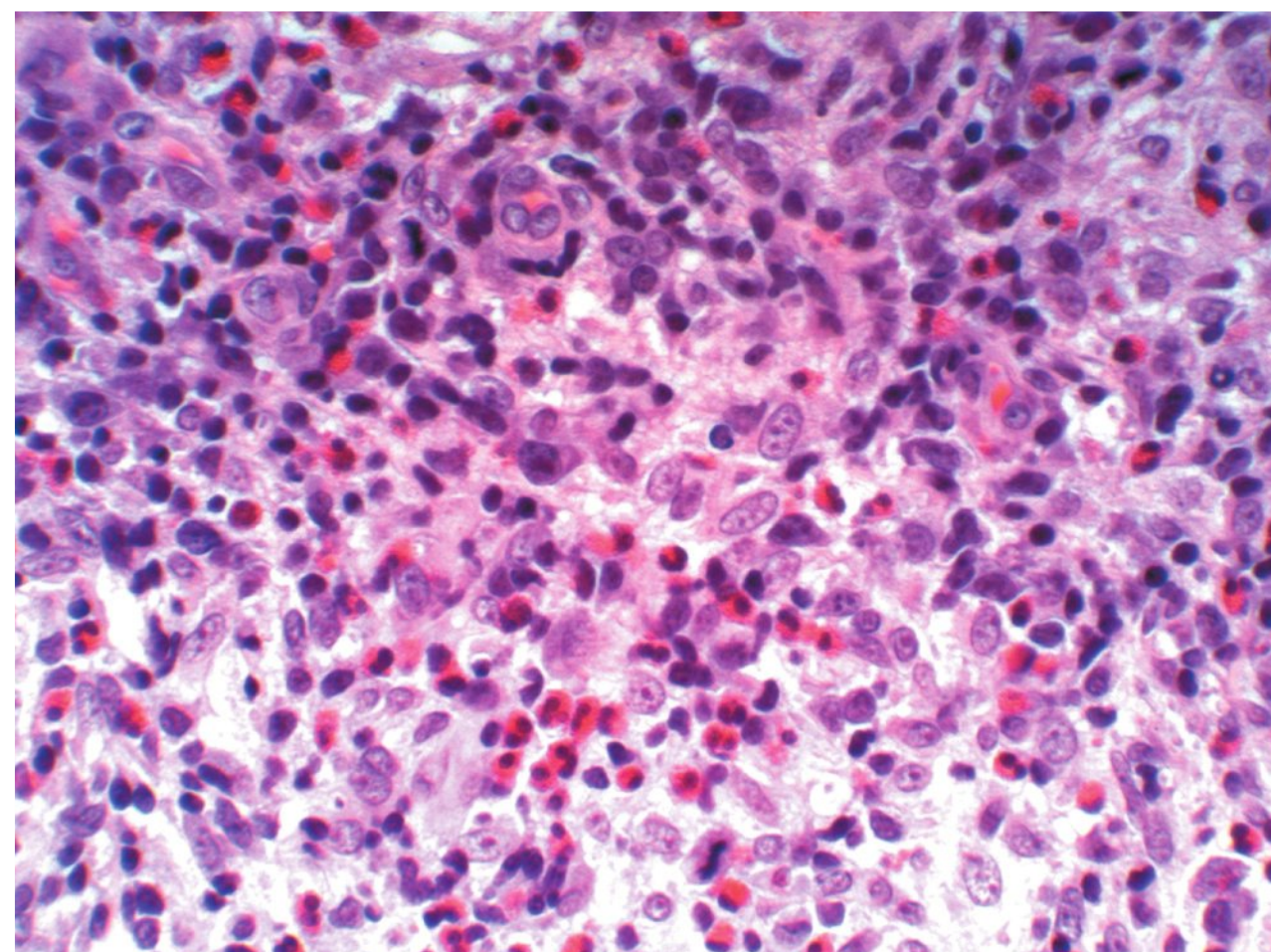


FIGURE 6.43.6 Lymph node biopsy of lymphoepithelioid lymphoma shows large numbers of epithelioid histiocytes and eosinophils intermixing with lymphoma cells. Hematoxylin and eosin, 50× magnification.

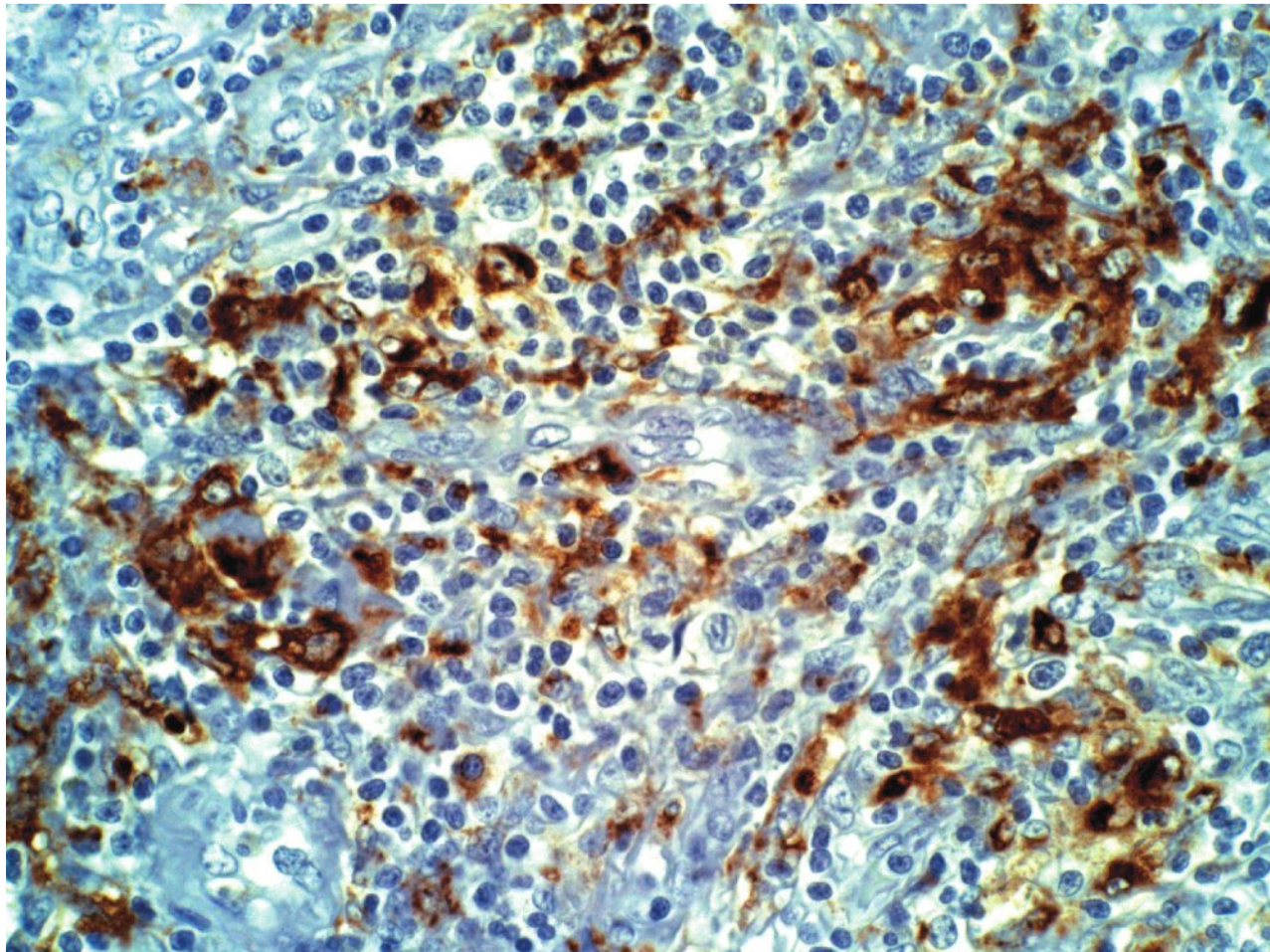


FIGURE 6.43.7 Lymph node biopsy of lymphoepithelioid lymphoma shows numerous histiocytes demonstrated by CD68 stain. Immunoperoxidase, 40× magnification.

of CD43 alone is not sufficient to ascertain a T-cell lineage, as it is also a myeloid marker and can be demonstrated in myeloid sarcoma. CD43 can also be coexpressed with B-cell antigens in some B-cell lymphomas. CD20 stain can help to diagnose T-cell/histiocyte-rich B-cell lymphoma, but the demonstration of CD20 does not exclude T-cell lymphoma, as a rare type of CD20-positive T-cell lymphoma is present (30,31). In some cases of PTCL-U, B-cell proliferation may be present and may lead to the misdiagnosis of B-cell lymphoma (11). Unfortunately, in situ hybridization for *k* and *l* RNA and immunoglobulin heavy-chain gene rearrangement may not help to distinguish lymphoid hyperplasia from lymphoproliferative disorder in this setting (11). Furthermore, LEL of B-cell type has been reported (25). Some of these cases may represent histiocyte-rich B-cell lymphomas (32,33).

Epstein-Barr virus (EBV) can be demonstrated in up to 50% of PTCL-U cases by in situ hybridization (EBER),

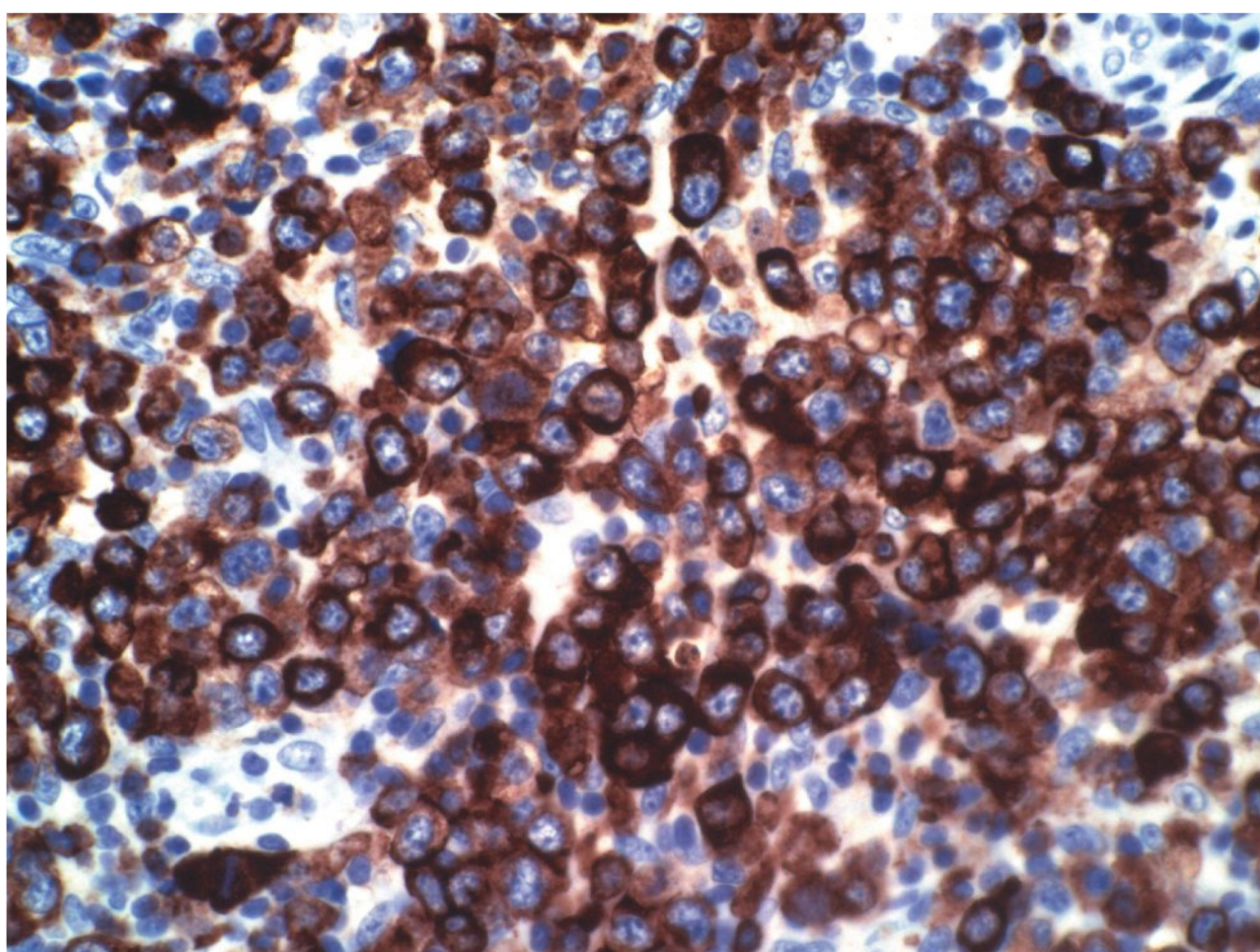


FIGURE 6.43.8 Lymph node biopsy of a PTCL shows exclusive CD4 staining on the tumor cells. Immunoperoxidase, 50× magnification.

usually on reactive B cells and rarely on scattered tumor cells (13). The EBV+ B-cells may assume the configuration of Reed-Sternberg-like cells, mimicking Hodgkin lymphoma (13). The presence of EBV is usually associated with a lower survival rate (13).

CD30+ and CD15+ are useful to identify the Reed-Sternberg cells in Hodgkin lymphoma. CD30+, CD15– immunophenotype is characteristic of anaplastic large-cell lymphoma, but can also be present in PTCL-U, particularly in the large-cell variant (4). In fact, even CD15 can be occasionally demonstrated in PTCL-U and predict a poor prognosis (18). The Reed-Sternberg-like cells are positive for CD15 in 10% of LEL cases (9). When an immunophenotype of CD30+, CD15– is identified, *Alk1* can be used to differentiate PTCL-U from anaplastic large-cell lymphoma.

Cytotoxic granule proteins (T-cell intracellular antigen [TIA]-1, and less frequently, granzyme B, and perforin) can be demonstrated in PTCL-U cases, especially those with a CD8+ phenotype, which had a positive rate of 32/38 cases as demonstrated in one study (28). CD56 can be present in rare cases (28). These cases should be distinguished from NK/NK-like T-cell lymphoma by TCR gene rearrangement and by killer immunoglobulin-like receptors study. Cytotoxic PTCL-U are generally associated with poor prognosis (11,13).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry is not as helpful as immunohistochemistry because there are no specific monoclonal markers for PTCL, and flow cytometric study lacks direct morphologic correlation. Immunohistochemical stains, in contrast, can demonstrate all pan-T-cell markers as well as the CD4 or CD8 subset on the tumor cells. The demonstration of a follicular helper T-cell phenotype is particularly useful for the diagnosis of the follicular variant. Some special stains can further differentiate the subtype of PTCL, such as CD15, CD30, CD16, CD56, CD57, and *Alk1*. Ki-67 may help to distinguish the neoplastic T-cell from the normal T-cell and predict a poor prognosis.

The current case presented with B symptoms, enlargement of multiple lymph nodes, and splenomegaly. Lymph node biopsy revealed a diffuse infiltrating small-cell lymphoma with numerous clusters of epithelioid histiocytes and eosinophilia. Immunophenotyping by both flow cytometry and immunohistochemistry demonstrated a predominant helper T-cell (CD4+) phenotype. The percentage of CD7 was lower than other pan-T-cell markers, as detected by flow cytometry, representing selective loss of T-cell antigen. Specific features of anaplastic large-cell lymphoma, AITL, and T-zone lymphoma were not present. Therefore, a diagnosis of LEL was established. Two unusual findings in this case were the development of a B-cell cutaneous lymphoma and amyloidosis in the skin. PTCL-U may have skin involvement, but a lymphoma of different cell lineage is seldom seen. Amyloidosis is also rarely seen in T-cell lymphomas, but there was no evidence of myeloma in this case.

Molecular Genetics

T-cell receptor (TCR) gene rearrangement is frequently demonstrated in PTCL-U, including LEL (16) and T-zone lymphoma (18). The positive rate for TCR gene rearrangement

in LEL varies from 56% to 100% (15,16,34,35). A false-negative result is probably due to the existence of a low percentage of tumor cells, which can be roughly estimated by the percentage of CD4/Ki-67-positive cells (16). Immunoglobulin heavy-chain gene rearrangement is usually negative in PTCL-U cases. Specific cytogenetic abnormalities have been detected in several PTCL subtypes, such as t(2;5) in anaplastic large T-cell lymphoma and isochromosome 7q in hepatosplenic T-cell lymphoma (9). In PTCL-U cases, trisomy 3, 6q-, trisomy 7q, and monosomy 13 have been reported (9). In a study of seven cases of LEL, six showed cytogenetically abnormal clones, always including aberration of chromosome 3 (36). In those cases, 3q22 is either broken or duplicated. Another study found cytogenetic abnormalities in 13 of 18 cases of LEL; most cases showed trisomy 3 or trisomy 5 (37). Trisomy 3 is also the most common aberration observed in T-zone lymphoma cases (38,39). A nonrandom karyotypic aberration, t(5;9)(q33;q22) representing SYK-ITK fusion, has been recently identified in the follicular variant (40).

In a study of 36 PTCL-U cases with comparative genomic hybridization, recurrent chromosomal losses were found on chromosome 13q (36%), 6q and 9p (31% each), 10q and 12q (28% each), and 5q (25%) (41). Recurrent gains were found on chromosome 7q22 (31%) (41). Another study found recurrent gains in chromosomes 7q, 8q, 17q, and 22q, and recurrent losses in chromosomes 4q, 5q, 6q, 9q, 10q, 12q, and 13q (42). A study with array comparative genomic hybridization analysis identified a subgroup of PTCL-U cases with genomic imbalance, which expresses CCR4 and is morphologically similar to that of lymphoma-type adult T-cell leukemia/lymphoma (43). These two entities also shared many regions of genomic alterations and showed similar overall survival curves (43). In contrast, PTCL-U cases without genomic imbalance showed a better prognosis than those with genomic imbalance (43).

Using DNA microarrays containing 6,386 cancer-related genes, Martinez-Delgado et al. (44) were able to identify the specific gene expression profile in PTCL that was distinguished from that of the lymphoblastic lymphoma. These authors also found a close relationship between genes associated with survival and those that differentiate the stages of disease and the responses to therapy.

A study of the nuclear factor- κ B (NF- κ B) using complementary DNA (cDNA) microarrays in a group of 62 PTCL patients found that one third of PL cases showed clearly reduced NF- κ B expression, whereas another group showed high expression of these genes (45). Patients with reduced expression of NF- κ B genes were associated with shorter survival; this finding was an independent prognostic factor in a multivariate analysis. Furthermore, gene expression profiling (GEP) is also able to distinguish PTCL-U from AITL, activated CD4+ from CD8+ profile, and CD30+ from CD30- PTCL-U cases (46). In addition, GEP can delineate prognostic groups depending on the proliferative signature and suggest therapeutic options regarding the regulation of NF- κ B, the use of CD52 as target for immunotherapy, or the use of kinase inhibitors for platelet-derived growth factor receptor α (46).

The salient features of laboratory diagnosis of PTCL are summarized in Table 6.43.3.

TABLE 6.43.3

Salient Features for Laboratory Diagnosis of PTCL-U

1. Most tumors are of helper T-cell type (CD3+, CD4+, CD8-), but lymphoepithelioid lymphoma is predominant with cytotoxic/suppressor T-cell type (CD3+, CD4-, CD8+). Follicular variant shows a follicular helper T-cell phenotype (CD3+, CD4+, BCL-6+, CXCL13+, PD-1+)
2. Selective loss of pan-T-cell antigens (seldom seen in lymphoepithelioid lymphoma)
3. No immature T-cell markers (CD1a-, TdT-)
4. The Reed-Sternberg-like cells are usually negative for both CD15 and CD30, but can be positive for CD15 or CD30 in occasional cases.
5. TCR gene rearrangement is seen in most cases except for those with low percentage of CD4/Ki-67 cells.
6. Aberration of chromosome 3, especially trisomy 3, is common. Follicular variant may show t(5;9)(q33;q22)

PTCL-U, peripheral T-cell lymphoma, not otherwise specified; TdT, terminal deoxynucleotidyl transferase; TCR, T-cell receptor.

Clinical Manifestations

A study of 108 cases of LEL showed that the median age was 60 years, with the range from 21 to 87 (47). The male/female ratio of the same series was 1.3:1. The initial clinical symptoms included lymphadenopathy (100%), splenomegaly (43%), hepatomegaly (23%), B symptoms (60%), pruritus (18%), skin rash (7%), fatigue (55%), and lymph node pain (17%) (14,33). At presentation, most patients were in advanced stages (stage I, 9%; stage II, 19%; stage III, 35%; and stage IV, 37%). However, bone marrow was rarely involved (5%) in this series, whereas five of nine cases had bone marrow involvement in other series (14). Extranodal manifestation, which is characteristic for other PTCLs (14), was not common in LEL. For instance, only 4% involved the skin, 4% soft tissue, 3% lung, and 1% each for pleura and stomach (47). In hematologic workup, anemia, lymphocytopenia, eosinophilia, and monocytosis were seen in 26% to 37% of cases (17,47). About one half of the cases showed hypoproteinemia and hypoalbuminemia. Hypogammaglobulinemia and hypergammaglobulinemia were seen in about one quarter of the cases, but no monoclonal gammopathy was detected in the series of 108 patients (47).

Patients with T-zone lymphoma are mostly in the fifth and sixth decades of life, with a male/female ratio of 1.5:1 (18). The clinical presentation is a rapid enlargement of lymph nodes, spleen, and liver (18). Institutional symptoms include malaise, fever, and sometimes skin rashes (19). However, lymphomatous infiltrate of the skin is seldom seen (18). Lung and pleura are often affected, but peripheral blood involvement is rare.

The overall survival for patients with LEL was 16 months (range, 1 to 49 months), with a 3-year survival of 36% (47). Those patients in stage I/II had a median survival of 18 months and a 3-year survival of 49% (47). A recent study found that CD8+LEL showed a significantly better overall survival than other CD8+ PTCL-U cases (28). The PTCL-U group, as a whole, has one of the lowest overall and failure-free survival rates (48). Patients with PTCL-U generally fare worse than those with diffuse large B-cell lymphoma in several studies (49). In a study of 385 PTCL-U cases, age >60 years, increased serum lactate dehydrogenase, ECOG performance status >2, and bone marrow involvement were found to be associated with a worse overall survival on multivariate analysis (50). Another group used age, performance status, lactate dehydrogenase, and Ki-67 marking >80% tumor cells to stratify three groups of patients with different responses to therapy and life expectancy (27).

REFERENCES

1. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classification of non-Hodgkin's lymphomas. *Cancer*. 1982;49:2112–2135.
2. Stansfeld AG, Diebold J, Kapanci Y, et al. Updated Kiel classification for lymphomas. *Lancet*. 1988;1:292–293.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms. A proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
4. Raifkiaer E, Müller-Hermelink HK, Jaffe ES. Peripheral T-cell lymphoma, unspecified. In: Jaffe ES, Harris NL, Stein H, et al., eds. *Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2002:227–229.
5. Pileri SA, Weisenburger DD, Sng I, et al. Peripheral T-cell lymphoma, not otherwise specified. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:306–308.
6. Chan JKC. Peripheral T-cell and NK-cell neoplasms: an integrated approach to diagnosis. *Mod Pathol*. 1999;12:177–199.
7. Ascani S, Zinzani PL, Gherlinzoni F, et al. Peripheral T-cell lymphomas. Clinico-pathologic study of 168 cases diagnosed according to the R.E.A.L. classification. *Ann Oncol*. 1997;8:583–592.
8. Rudiger T, Weisenburger DD, Anderson JR, et al. Peripheral T-cell lymphoma (excluding anaplastic large-cell lymphoma): results from the Non-Hodgkin's Lymphoma Classification Project. *Ann Oncol*. 2002;13:140–149.
9. Pinkus GS, Said JW. Peripheral T-cell lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:1091–1125.
10. Patsouris E, Noel H, Lennert K. Histological and immunological findings in lymphoepithelioid cell lymphoma (Lennert's lymphoma). *Am J Surg Pathol*. 1988;12:341–350.
11. Warnke RA, Jones D, His ED. Morphologic and immunophenotypic variants of nodal T-cell lymphomas and T-cell lymphoma mimics. *Am J Clin Pathol*. 2007;127:511–527.
12. Dogan A, Morice WG. Bone marrow histopathology in peripheral T-cell lymphomas. *Br J Haematol*. 2004;127:140–154.
13. de Leval L, Ralfkiaer E, Jaffe ES. Peripheral T-cell lymphoma, not otherwise specified. In: Jaffe ES, Harris NL, Vardiman JW, et al., eds. *Hematopathology*. Philadelphia, PA: Elsevier; 2011:541–553.
14. Pinkus GS, O'Hara CJ, Said JW. Peripheral/post-thymic T-cell lymphomas: a spectrum of disease. Clinical, pathologic, and immunologic features of 78 cases. *Cancer*. 1990;65:971–998.
15. Nakamura S, Suchi T. A clinicopathologic study of node-based, low-grade, peripheral T-cell lymphoma. Angioimmunoblastic lymphoma, T-zone lymphoma, and lymphoepithelioid lymphoma. *Cancer*. 1991;67:2565–2578.
16. Takagi N, Nakamura S, Ueda R, et al. A phenotypic and genotypic study of three node-based, low grade peripheral T-cell lymphomas: angioimmunoblastic lymphoma, T-zone lymphoma, and lymphoepithelioid lymphoma. *Cancer*. 1992;69:2571–2582.
17. Patsouris E, Noel H, Lennert K. Angioimmunoblastic lymphadenopathy-type T-cell lymphoma with a high content of epithelioid cells: histopathology and comparison with lymphoepithelioid lymphoma. *Am J Surg Pathol*. 1989;13:262–275.
18. Kazakov DV, Kempf W, Michaelis S, et al. T-zone lymphoma with cutaneous involvement: a case report and review of the literature. *Br J Dermatol*. 2002;146:1096–1100.
19. Such T, Lennert K, Tu LY, et al. Histopathology and immunohistochemistry of peripheral T-cell lymphomas: a proposal for their classification. *J Clin Pathol*. 1987;40:995–1015.
20. de Leval L, Savilo E, Longtine J, et al. Peripheral T-cell lymphoma with follicular involvement and a CD4+/bcl-6+ phenotype. *Am J Surg Pathol*. 2001;25:395–400.
21. Macon WR, Williams ME, Greer JP, et al. Paracortical nodular T-cell lymphoma. Identification of an unusual variant of peripheral T-cell lymphoma. *Am J Surg Pathol*. 1995;19:297–303.
22. Uherova P, Ross CW, Finn WG, et al. Peripheral T-cell lymphoma mimicking marginal zone B-cell lymphoma. *Mod Pathol*. 2002;15:420–425.
23. Rudiger T, Ichinohasama R, Ott MM, et al. Peripheral T-cell lymphoma with distinct perifollicular growth pattern. A distinct subtype of T-cell lymphoma? *Am J Surg Pathol*. 2000;24:117–122.
24. Ikonomou IM, Tierens A, Troen G, et al. Peripheral T-cell lymphoma with involvement of the expanded mantle zone. *Virchows Arch*. 2006;449:78–87.
25. Spier CM, Lippman SM, Miller TP, et al. Lennert's lymphoma: a clinicopathologic study with emphasis on phenotype and its relationship to survival. *Cancer*. 1988;61:517–524.
26. Chott A, Augustin I, Wrba F, et al. Peripheral T-cell lymphomas: a clinicopathologic study of 75 cases. *Hum Pathol*. 1990;21:1117–1125.
27. Went P, Agostinelli C, Gallamini A, et al. Marker expression in peripheral T-cell lymphoma: a proposed clinical-pathologic prognostic score. *J Clin Oncol*. 2006;24:2472–2479.
28. Geissinger E, Odenwald T, Lee SS, et al. Nodal peripheral T-cell lymphomas and, in particular, their lymphoepithelioid (Lennert's) variant are often derived from CD8+ cytotoxic T-cells. *Virchows Arch*. 2004;445:334–343.
29. Rodriguez-Pinilla SM, Atienza L, Murillo C, et al. Peripheral T-cell lymphoma with follicular T-cell markers. *Am J Surg Pathol*. 2008;32:1787–1799.
30. Yao X, Teruya-Feldstein J, Raffeld M, et al. Peripheral T-cell lymphoma with aberrant expression of CD79a and CD20: a diagnostic pitfall. *Mod Pathol*. 2001;14:105–110.
31. Sun T, Akalin A, Rodacker M, et al. CD20 positive T cell lymphoma: is it a real entity? *J Clin Pathol*. 2004;57:442–444.
32. Delabie J, Vandenberghe E, Kennes C, et al. Histiocyte-rich B-cell lymphoma: a distinct clinicopathologic entity possibly related to lymphocyte predominant Hodgkin's disease, paragranuloma subtype. *Am J Surg Pathol*. 1992;16:37–48.
33. Sun T, Susin M, Tomao FA, et al. Histiocyte-rich B-cell lymphoma. *Hum Pathol*. 1997;28:1321–1324.
34. O'Connor NTJ, Feller AC, Asinscoat JS, et al. T-cell origin of Lennert's lymphoma. *Br J Haematol*. 1986;64:521–528.

35. Feller AC, Griesser GH, Mak TW, et al. Lymphoepithelioid lymphoma (Lennert's lymphoma) is a monoclonal proliferation of helper/inducer T-cells. *Blood*. 1986;68:663–667.
36. Godde-Salz E, Feller AC, Lennert K. Cytogenetic and immunohistochemical analysis of lymphoepithelioid cell lymphoma (Lennert's lymphoma): further substantiation of its T-cell nature. *Leuk Res*. 1985;10:313–323.
37. Godde-Salz E, Feller AC, Lennert K. Chromosomes in Lennert's lymphoma. *Leuk Res*. 1986;11:181–190.
38. Schlegelberger B, Himmler A, Godde E, et al. Cytogenetic findings in peripheral T-cell lymphomas as a basis for distinguishing low-grade and high-grade lymphomas. *Blood*. 1994;83:505–511.
39. Godde-Salz E, Schwarze EW, Stein H, et al. Cytogenetic findings in T-zone lymphoma. *J Cancer Res Clin Oncol*. 1981;101:81–89.
40. Streubel B, Vinatzer V, Willheim M, et al. Novel t(5;9) (q33;q22) fuses ITK to SYK in unspecified peripheral T-cell lymphoma. *Leukemia*. 2006;20:313–318.
41. Zettl A, Rudiger T, Konrad MA, et al. Genomic profiling of peripheral T-cell lymphoma, unspecified, and anaplastic large T-cell lymphoma delineates novel recurrent chromosomal alterations. *Am J Pathol*. 2004;164:1837–1848.
42. Rizvi MA, Evens AM, Tallman MS, et al. T-cell non-Hodgkin lymphoma. *Blood*. 2006;107:1255–1264.
43. Nakagawa M, Nakagawa-Oshiro A, Karnan S, et al. Array comparative genomic hybridization analysis of PTCL-U reveals a distinct subgroup with genetic alterations similar to lymphoma-type adult T-cell leukemia/lymphoma. *Clin Cancer Res*. 2009;15:30–38.
44. Martinez-Delgado B, Melendex B, Cuadros M, et al. Expression profiling of T-cell lymphomas differentiates peripheral and lymphoblastic lymphomas and defines survival related genes. *Clin Cancer Res*. 2004;10:4971–4982.
45. Martinez-Delgado B, Cuadros M, Honrado E, et al. Differential expression of NF-kappaB pathway genes among peripheral T-cell lymphoma. *Leukemia*. 2005;19:2254–2263.
46. Costello R, Sanchez C, Le Treut T, et al. Peripheral T-cell lymphoma gene expression profiling and potential therapeutic exploitations. *Br J Haematol*. 2009;150:21–27.
47. Patsouris E, Engelhard M, Zwingers T, et al. Lymphoepithelioid cell lymphoma (Lennert's lymphoma): clinical features derived from analysis of 108 cases. *Br J Haematol*. 1993;84:346–348.
48. The Non-Hodgkin's lymphoma classification Project. A clinical evaluation of the International Lymphoma study Group classification of non-Hodgkin's lymphoma. *Blood*. 1997;89:3909–3918.
49. Moribito F, Gallamini A, Stelitano C, et al. Clinical relevance of immunophenotype in a retrospective comparative study of 297 peripheral T-cell lymphomas, unspecified, and 496 diffuse large B-cell lymphomas. *Cancer*. 2004;101:1601–1608.
50. Gallamini A, Stelitano C, Calvi R, et al. Peripheral T-cell lymphoma unspecified (PTCL-U): a new prognostic model from a retrospective multicentric clinical study. *Blood*. 2004;103:2474–2479.

CASE 44

Angioimmunoblastic T-Cell Lymphoma

CASE HISTORY

A 60-year-old man presented with left leg edema for several weeks. Ultrasound examination for blood clot found inguinal adenopathy incidentally. Further CT scan of the chest and abdomen demonstrated diffuse adenopathy, including axillary, inguinal, periaortic, hilar, and mediastinal regions. He had lost 10 lb in last several months, but denied having fever, night sweats, and other constitutional symptoms. Physical examination revealed left leg edema and skin rash at left shin. Bilateral axillary and inguinal lymph nodes were enlarged. There was no palpable hepatosplenomegaly. Laboratory examination revealed a total leukocyte count of 3,800/mL, hemoglobin 12.5 g/dL, hematocrit 36%, and platelet count 200,000/mL. His blood chemistry profile was unremarkable except for a lactate dehydrogenase level of 193 IU/L. His gamma globulin was within normal limits. An axillary lymph node biopsy was performed and showed features of lymphoma (Figs. 6.44.1–6.44.3). The patient underwent several cycles of chemotherapy with initial improvement but gradually became refractory to treatment. He died 2 years after diagnosis.

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The axillary lymph node biopsy showed that the tumor cells were immunoreactive to CD3 (Fig. 6.44.4), CD4 (Fig. 6.44.5), CD10 (Fig. 6.44.6), and BCL-6 (Fig. 6.44.7), but were negative for CD8 (Fig. 6.44.8), CD20 (Fig. 6.44.9), CD30 (Fig. 6.44.10), and ALK1 (Fig. 6.44.11). An extrafollicular follicular dendritic meshwork was demonstrated by CD21 staining (Fig. 6.44.12).

DISCUSSION

Angioimmunoblastic T-cell lymphoma (AITCL) was initially considered to be an atypical lymphoid hyperplasia and a premalignant lesion with a potential of developing into T-cell lymphoma (1). The essential histologic features include (a) total or partial effacement of lymph node architecture by diffuse polymorphic cellular infiltration, (b) marked proliferation of arborizing small blood vessels lined by tall endothelial cells (high endothelial venules), and (c) interstitial deposition of periodic-acid Schiff-positive

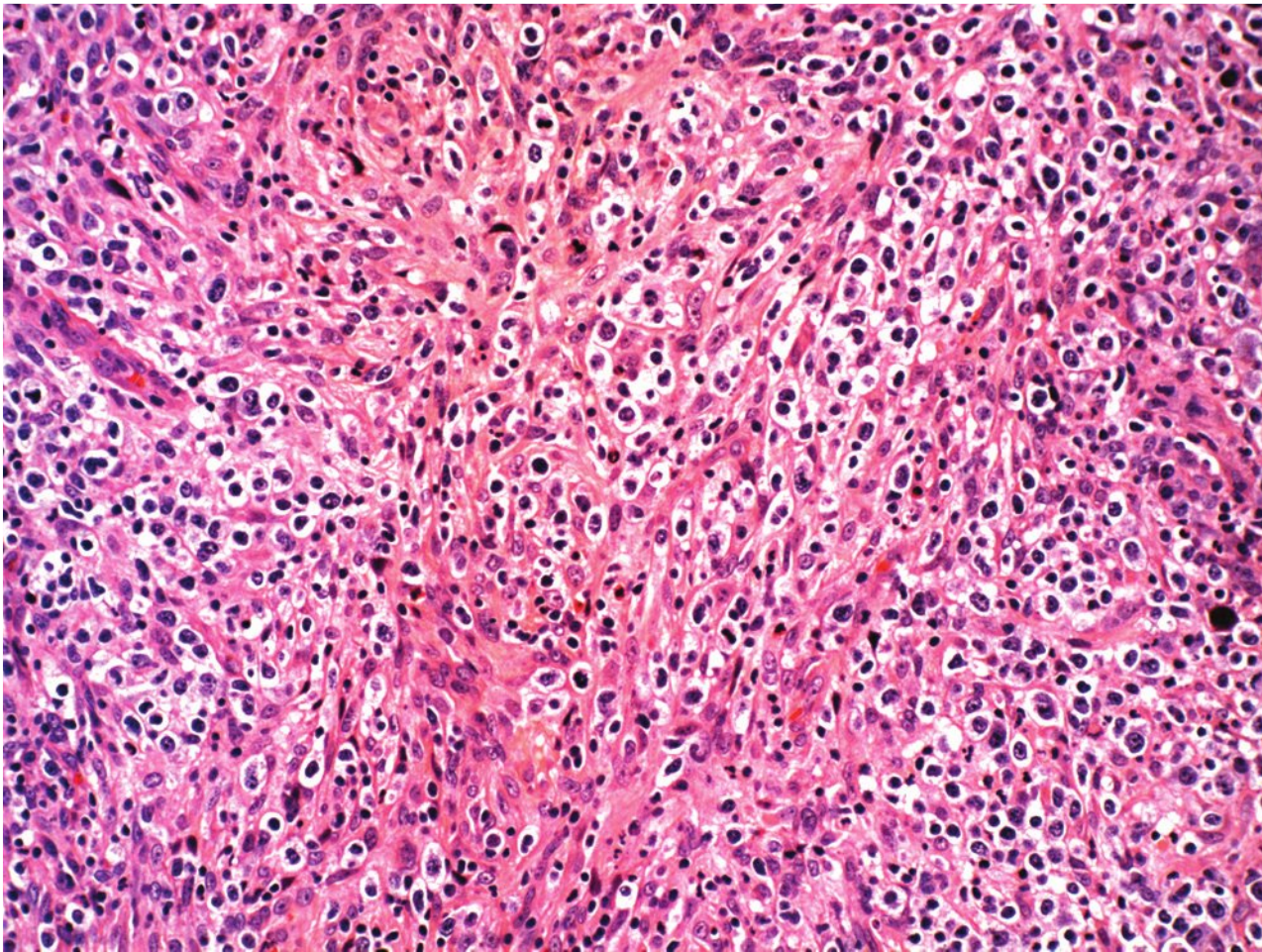


FIGURE 6.44.1 Lymph node biopsy shows effacement of normal architecture by arborizing blood vessels and large tumor cells with clear cytoplasm. H&E, $\times 20$.

material (2). Prizzera et al. (2) termed this condition as angioimmunoblastic lymphadenopathy with dysproteinemia. Subsequently, it was designated as immunoblastic lymphadenopathy by Lukes and Tindle (3), and lymphogranulomatosis X by Lennert (4). In spite of subtle differences between these three entities, they shared the same clinical features, such as generalized lymphadenopathy, hepatosplenomegaly, anemia, and hypergammaglobulinemia. With the advent of immunophenotypic and molecular genetic techniques, it finally became apparent that AITCL was a clonal T-cell neoplasm from the onset. AITCL is now recognized as a distinct entity by the World Health Organization classification (5).

AITCL is one of the common specific subtypes of peripheral T-cell lymphomas, accounting for 15% to 20% of cases (5). It constitutes approximately 1% to 2% of all non-Hodgkin lymphomas (5). However, this lymphoma was probably underdiagnosed because of the protean

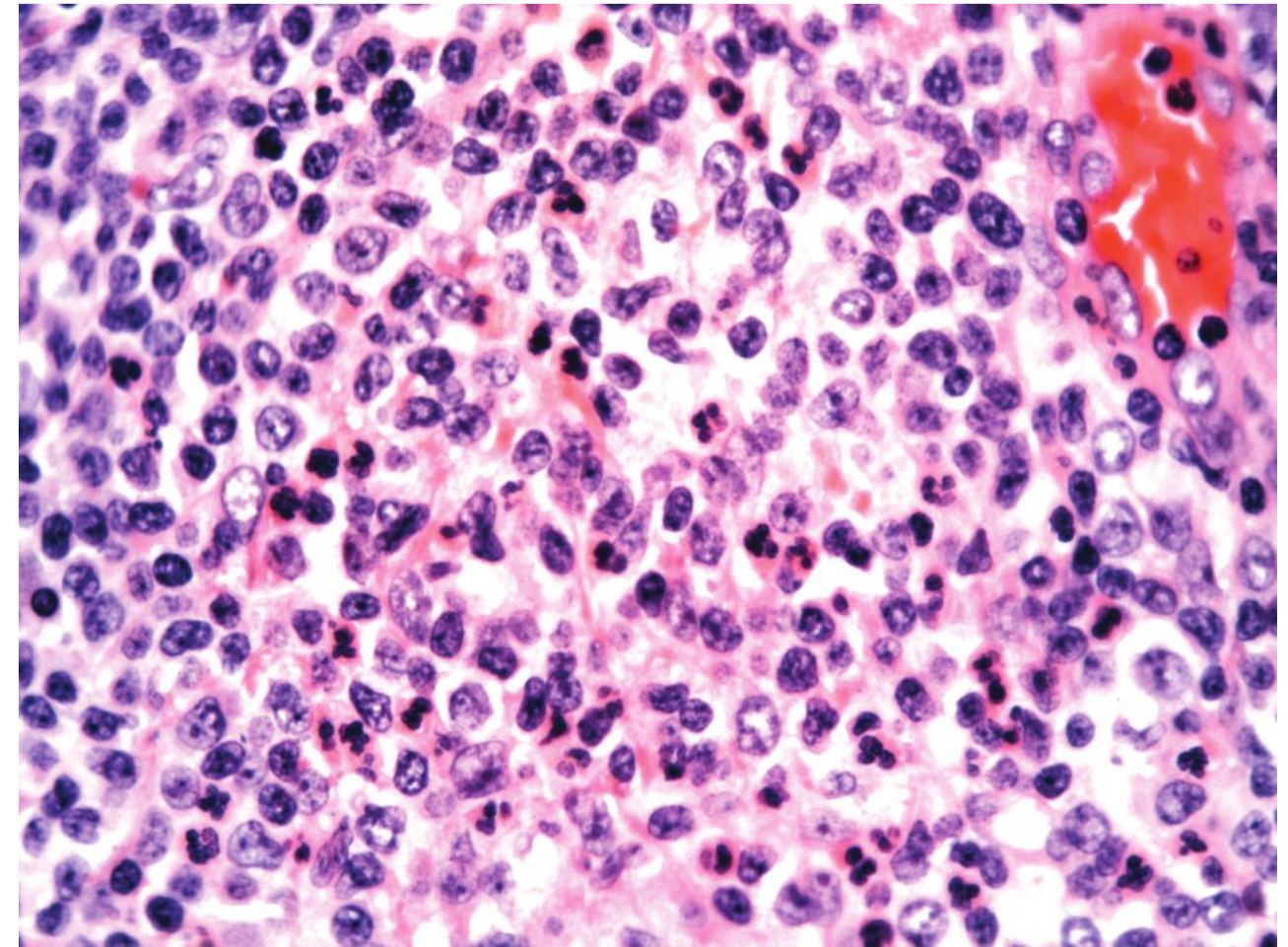


FIGURE 6.44.3 Higher magnification shows an area with mixed cellular infiltration. H&E, $\times 60$.

clinical presentation, partial preservation of lymph node architecture, and a mixed cellular infiltration, particularly in cases where the characteristic large tumor cells are in the minority.

Morphology

In AITCL, the lymph node architecture may be partially or completely effaced by a polymorphous infiltration in the paracortex that includes small lymphocyte, histiocytes, eosinophils, plasma cells, and immunoblasts (1,5–8). A few multinucleated giant cells or Reed-Sternberg–like cells may be seen in some cases. The tumor cells are of medium- to large-size with clear or pale cytoplasm and usually show a perivascular or a perifollicular distribution. The tumor cells may infiltrate the perinodal tissue, but the subcapsular sinuses remain patent. In the early stage, tumor cells may be rare. However, as the disease progresses, tumor cells become predominant and gradually replace the mixed

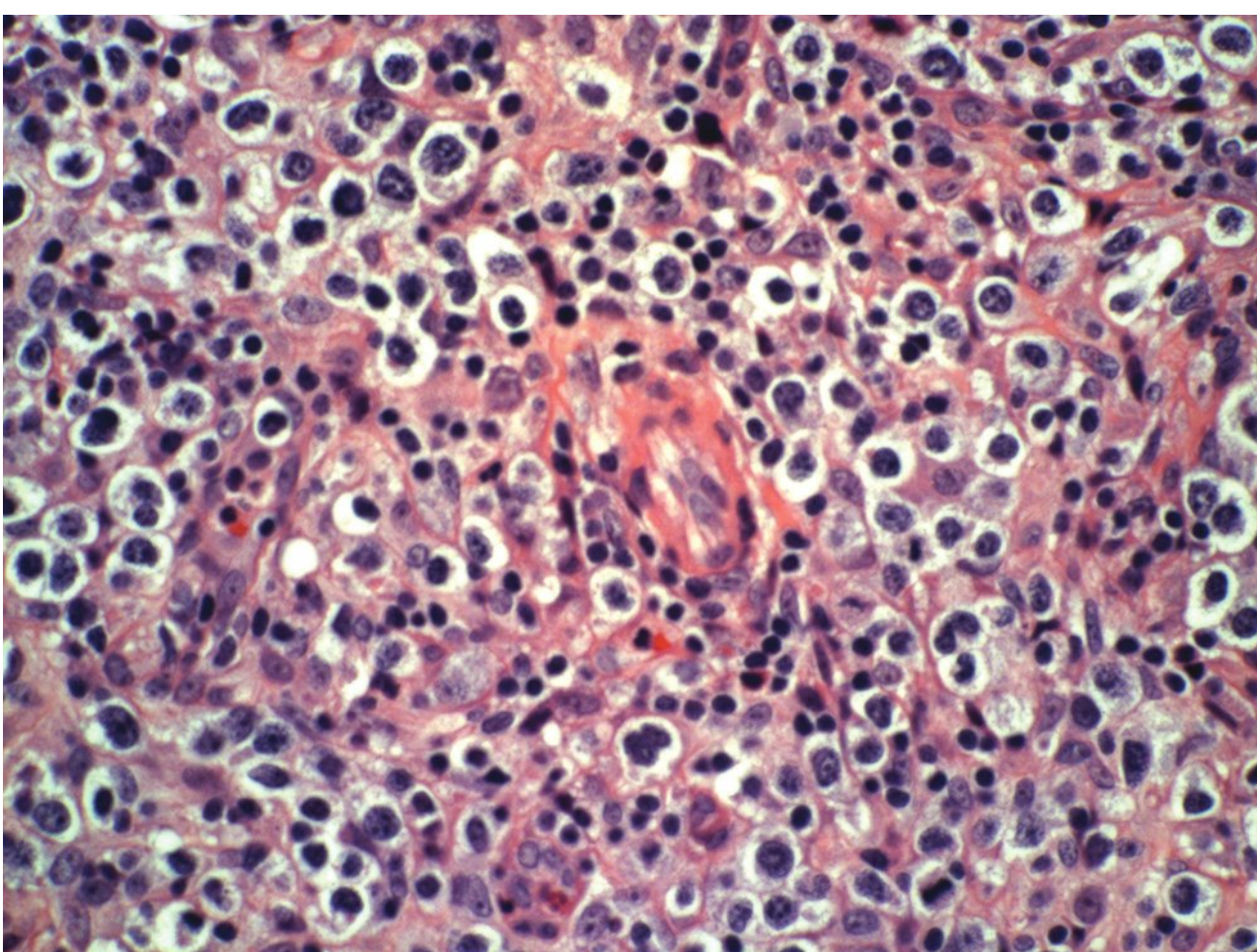


FIGURE 6.44.2 Higher magnification shows an area with predominantly clear large lymphoma cells. H&E $\times 40$.

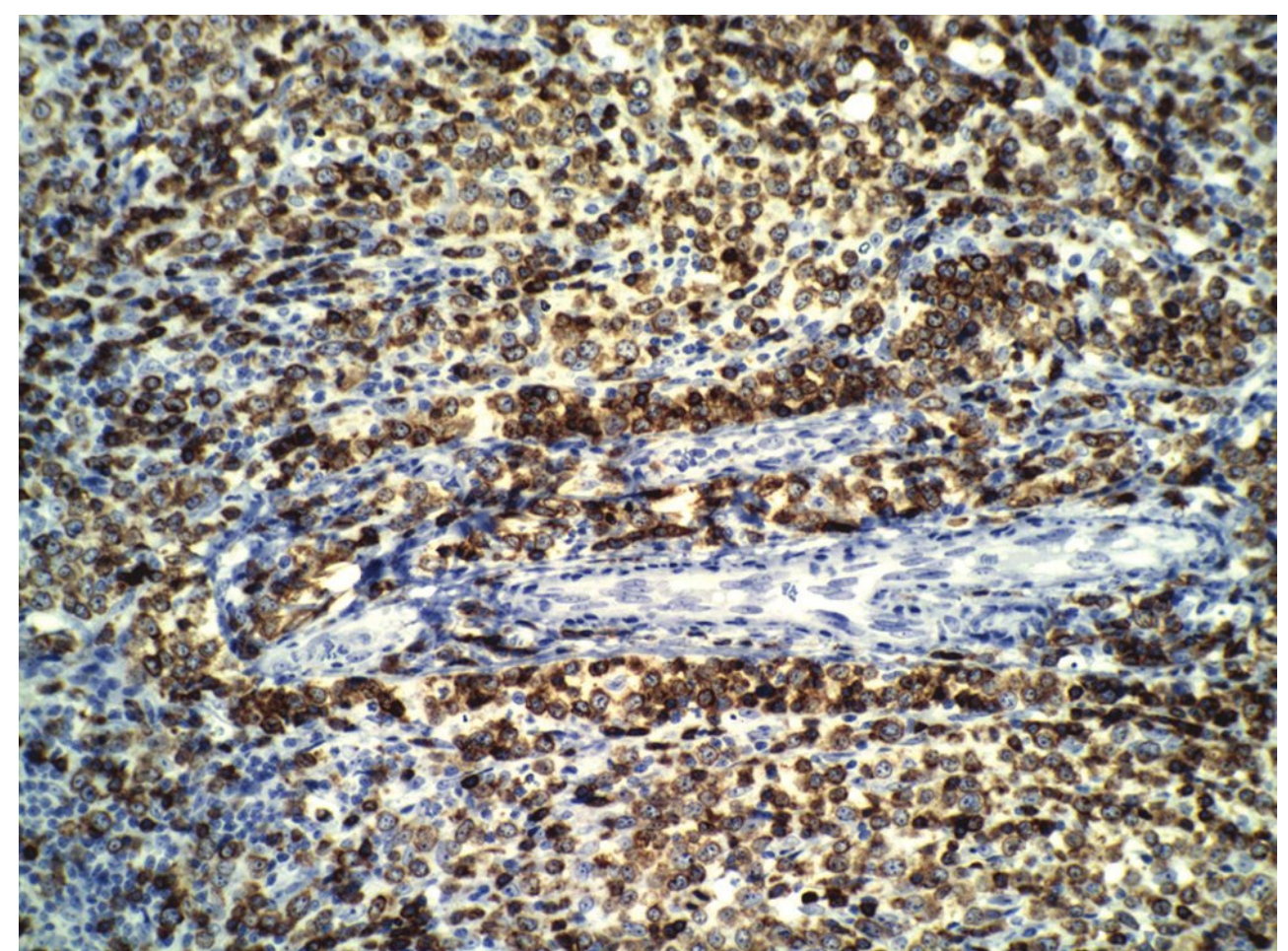


FIGURE 6.44.4 CD3 stain demonstrates T-lymphocytes around high endothelial venules. Immunoperoxidase, $\times 20$.

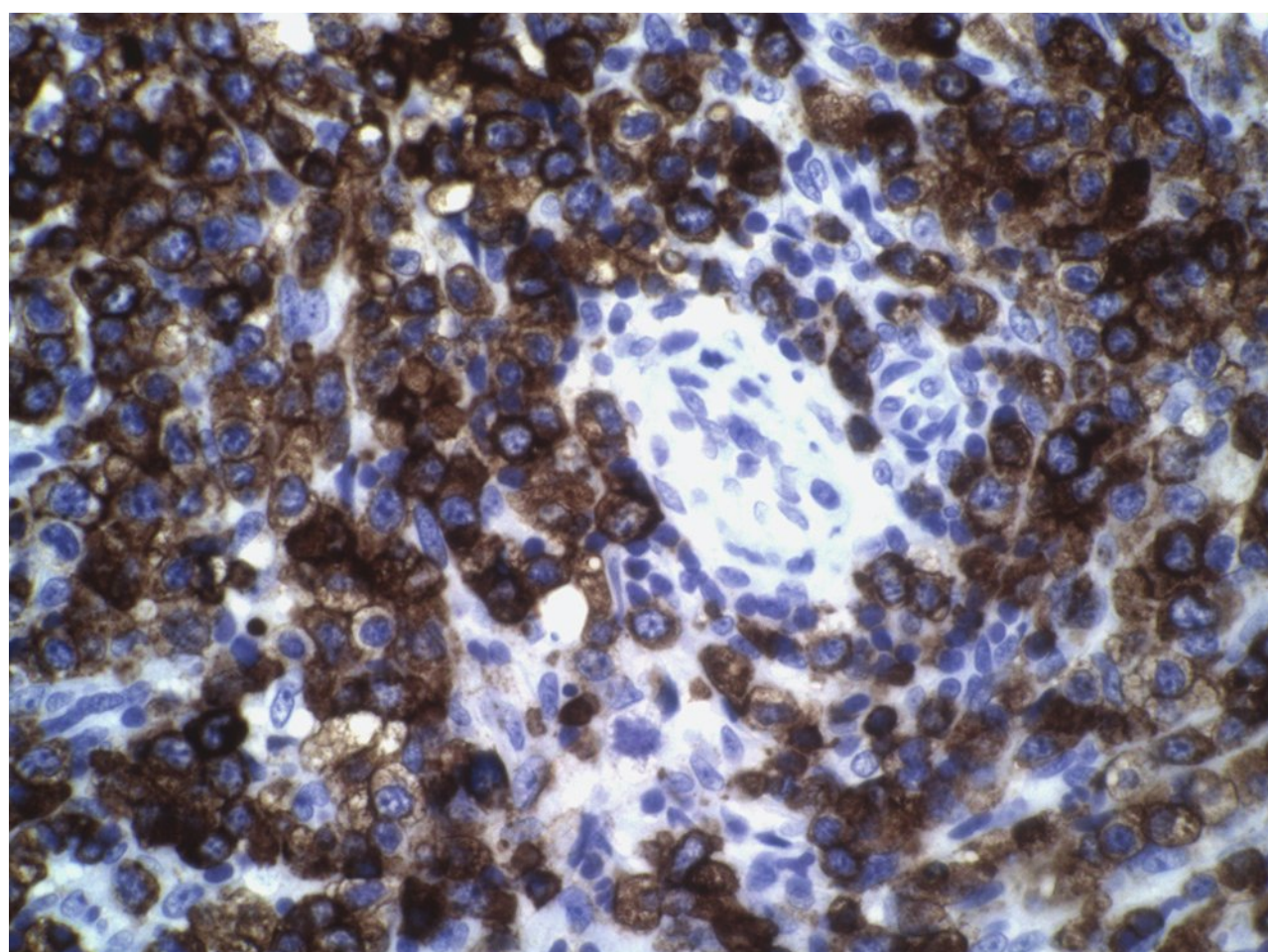


FIGURE 6.44.5 CD4 stain shows predominantly helper T-cells around a high endothelial venule. Immunoperoxidase, $\times 40$.

cellular population. The B-lymphocytes or the immunoblasts may also be increased in some cases. Those cells that are infected with Epstein-Barr virus (EBV) may finally transform into diffuse large B-cell lymphoma (9).

Another characteristic feature is vascular arborization, which is manifested as many branching and anastomosing blood vessels arranged in a haphazard pattern. Most of the blood vessels are high-endothelial venules. The third characteristic feature is proliferation of follicular dendritic cells (FDCs) in the paracortex that frequently surrounds the high endothelial venules.

However, it has been found that the histologic features are evolving in different stages. Attygalle et al. described three histologic patterns, which may represent different developmental stages of the disease (10). The major distinctions of these three stages are the gradual effacement of the normal lymph node architecture and the steady increase of FDCs. In pattern I, hyperplastic follicles with poorly developed mantles and ill-defined borders

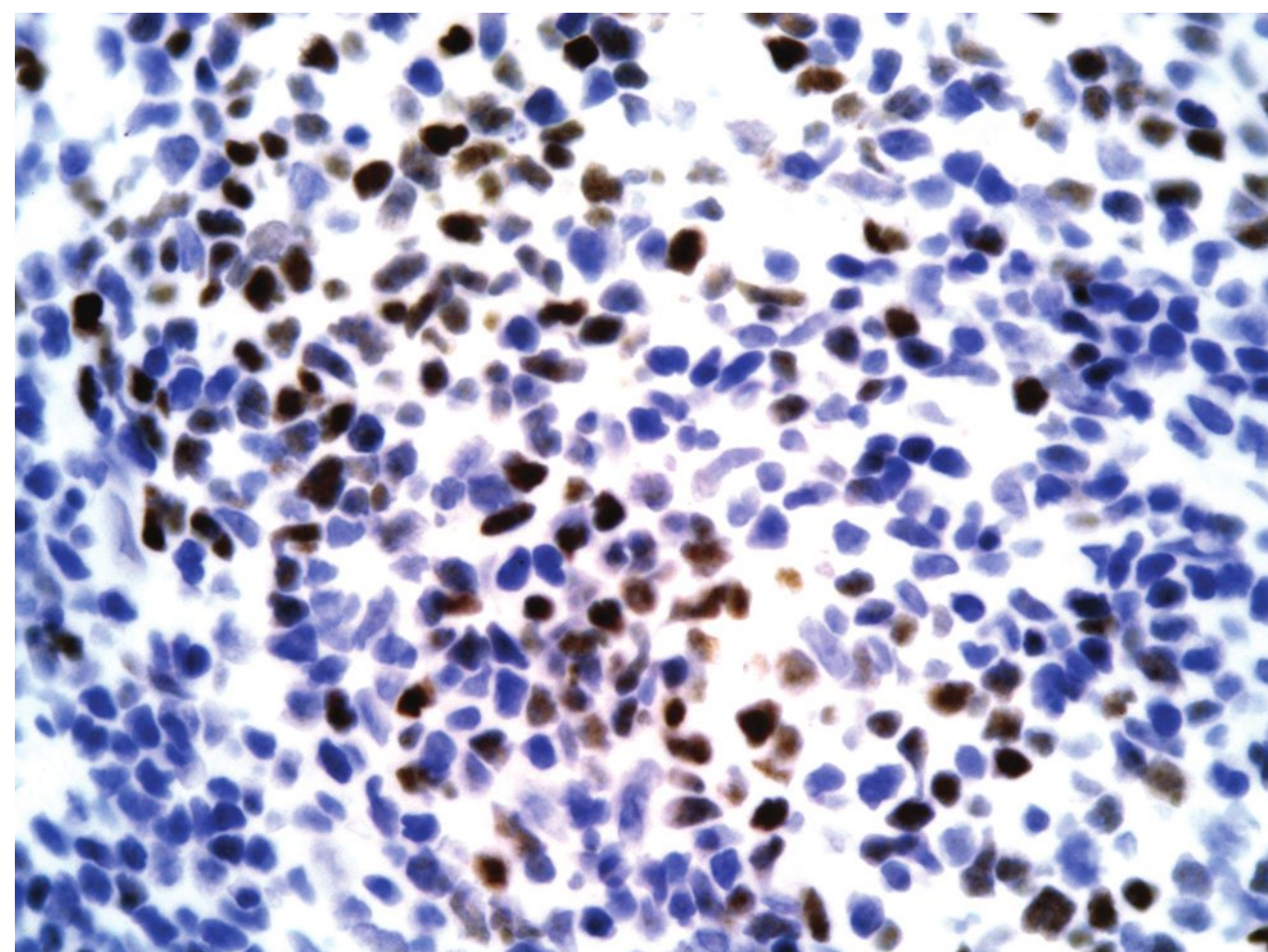


FIGURE 6.44.7 BCL-6 stain is positive in a fraction of lymphoma cells. Immunoperoxidase, $\times 60$.

are present without an extrafollicular FDC proliferation. Pattern II shows lymphocyte-depleted follicles with proliferation of FDCs extending beyond the follicles. Pattern III reveals total effacement of normal architecture with a prominent extrafollicular proliferation of FDCs. In each pattern, there is an expansion of the paracortex by a mixed cellular population.

Bone marrow is frequently (60% to 80%) involved in AITCL (11). The infiltration pattern is mostly nodular but can be interstitial or the existence of both patterns (11–13). Most cases show a paratrabeccular location of the infiltrates. The cellular component is usually polymorphous, and polyclonal plasmacytosis is also a common feature (11,13,14). While prominent vascular proliferation is frequently demonstrated in the bone marrow (12,13), FDC proliferation has seldom been detected (12).

The cutaneous lesion is often presented with dense, perivascular dermal lymphoid infiltrates. Four different histologic patterns have been reported in one study,

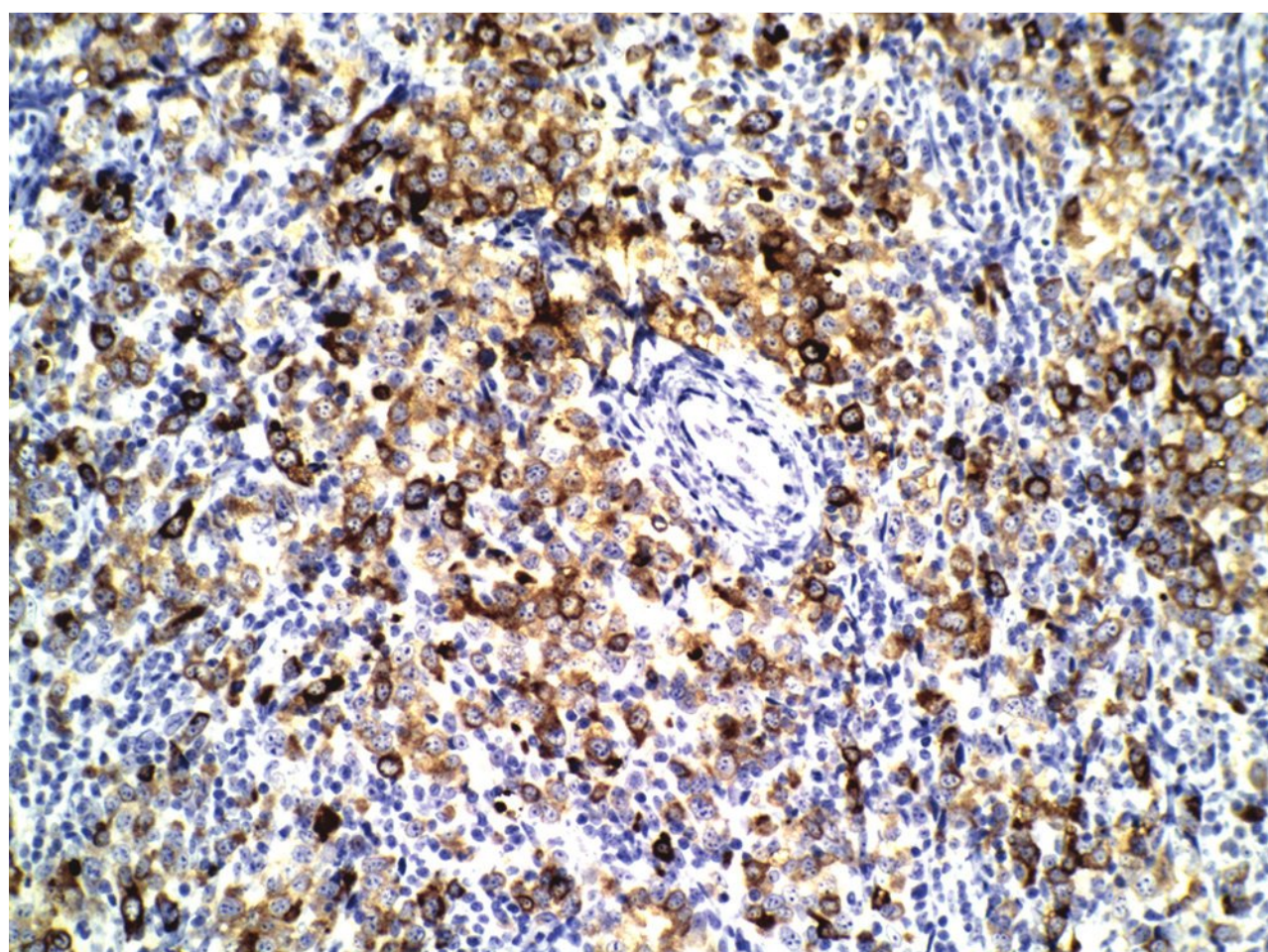


FIGURE 6.44.6 CD10 stain shows many positively stained tumor cells. Immunoperoxidase, $\times 20$.

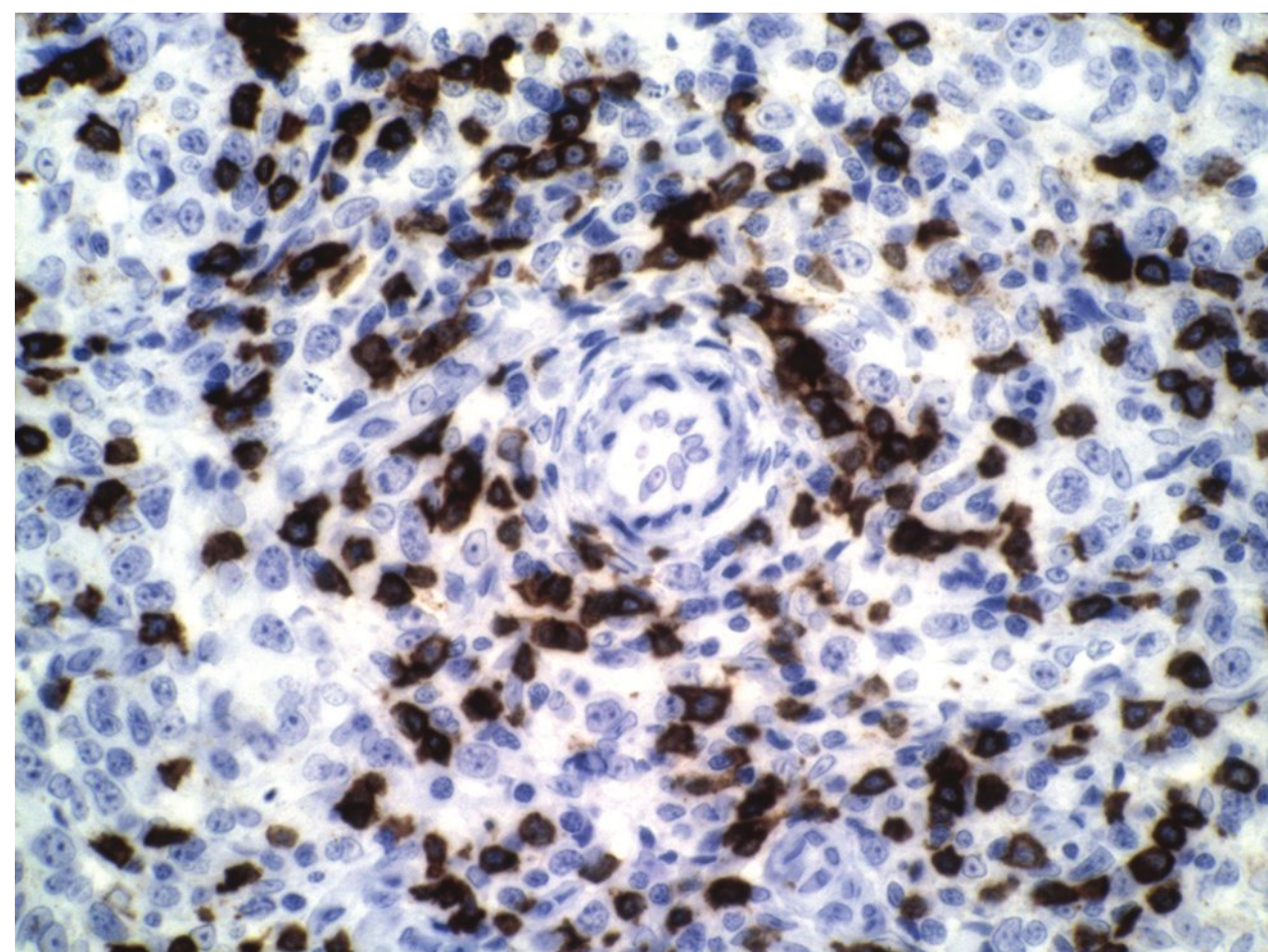


FIGURE 6.44.8 CD8 stain identifies scattered reactive T lymphocytes. Immunoperoxidase, $\times 40$.

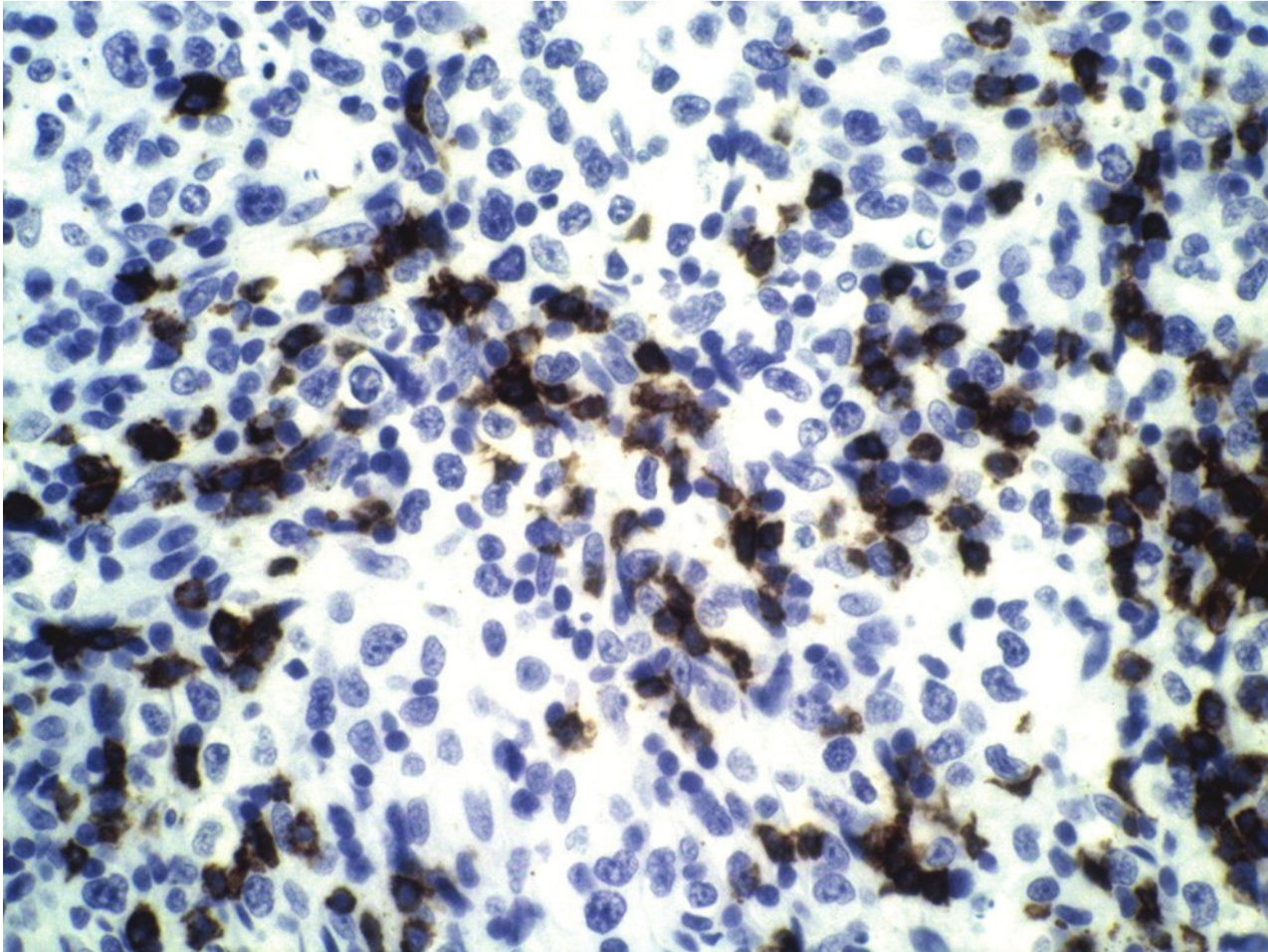


FIGURE 6.44.9 CD20 stain shows a few B lymphocytes. Immunoperoxidase, $\times 40$.

including (a) mild superficial perivascular lymphocytic infiltration without atypia; (b) sparse superficial perivascular infiltration with some atypical lymphoid cells and vascular hyperplasia; (c) dense, diffuse, superficial, and deep infiltrate of atypical lymphoid cells, associated with vascular hyperplasia; and (d) vasculitis without atypia of lymphoid cells (15).

The morphologic diagnostic criteria of AITCL are summarized in Table 6.44.1.

Immunophenotype

Immunophenotyping is very important in AITCL not only for an accurate diagnosis but also for the elucidation of the cell origin. As a T-cell lymphoma, all pan-T-cell antigens (CD2, CD3, CD5, and CD7) are demonstrated in AITCL. Unlike other T-cell tumors, “antigen loss” is seldom present (6). Most cases show a predominance of CD4 over CD8. However, this immunophenotype is also commonly seen

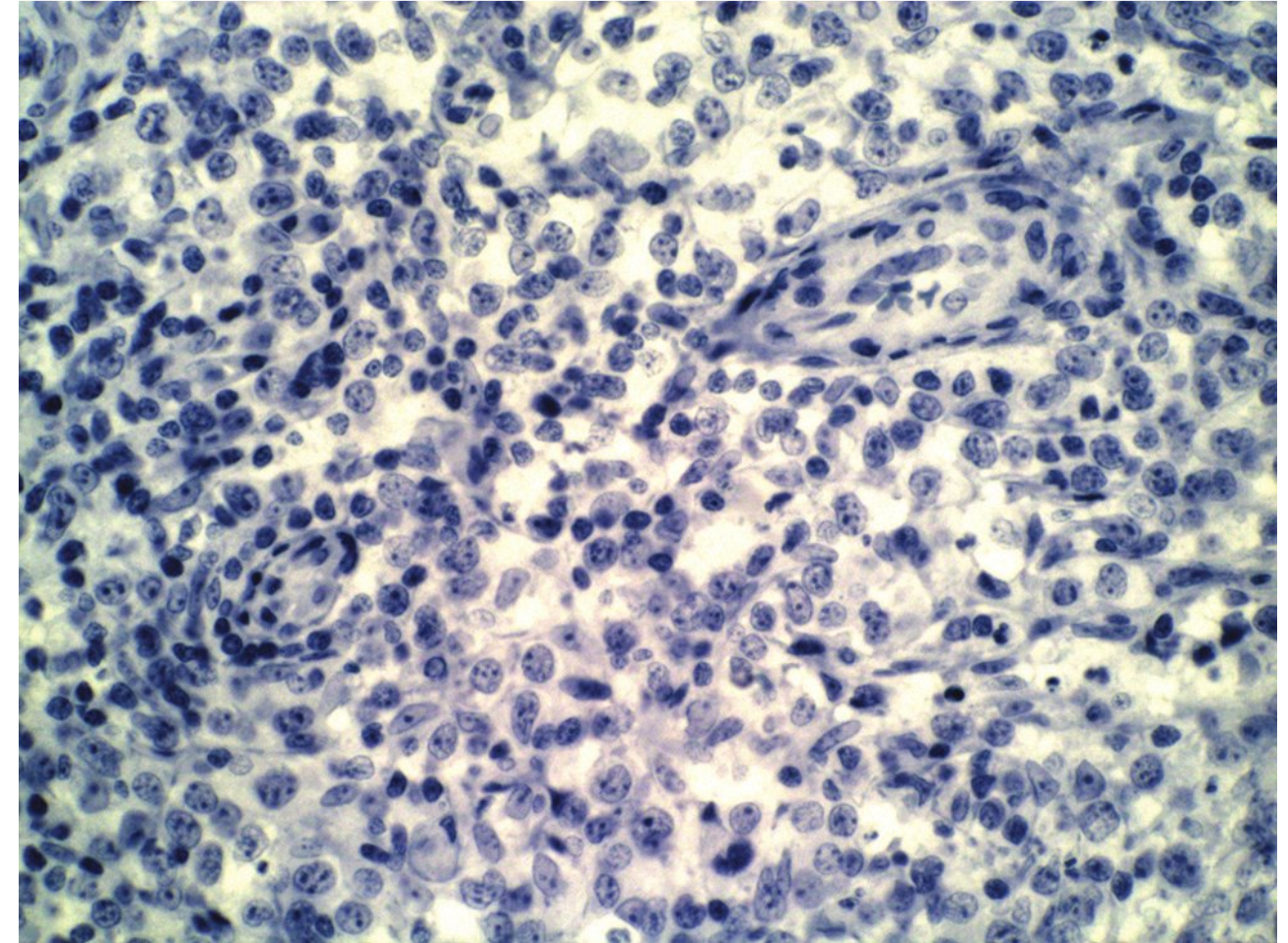


FIGURE 6.44.11 ALK1 stain reveals no positive staining. Immunoperoxidase, $\times 40$.

in other T-cell lymphomas. The first breakthrough is the identification of CD10 in the tumor cells that implicates a follicular origin and distinguishes AITCL from other T-cell tumor (10). The demonstration of BCL-6 on these tumor cells further confirms this hypothesis. Subsequently, several more markers have been identified that are specific for a subtype of T-helper cells, the follicular helper T-cell (T_{FH}) (16–18). These include the CXCL13 (a chemokine), programmed death-1 (PD1), and immune costimulatory molecule (ICOS), which are helpful for the diagnosis of AITCL in the lymph nodes (8,12,16). However, CXCL13 and CD10 are frequently negative or weakly positive in bone marrow specimens (11,12). BCL-6 with or without double staining with CD3 can help to identify tumor cells in the bone marrow, but PD1 only stains a small tumor population (11,12).

CXCL13 is particularly important in the pathogenesis of AITCL. It plays a role in B-cell recruitment and activation, which account for many of the B-cell related abnormalities,

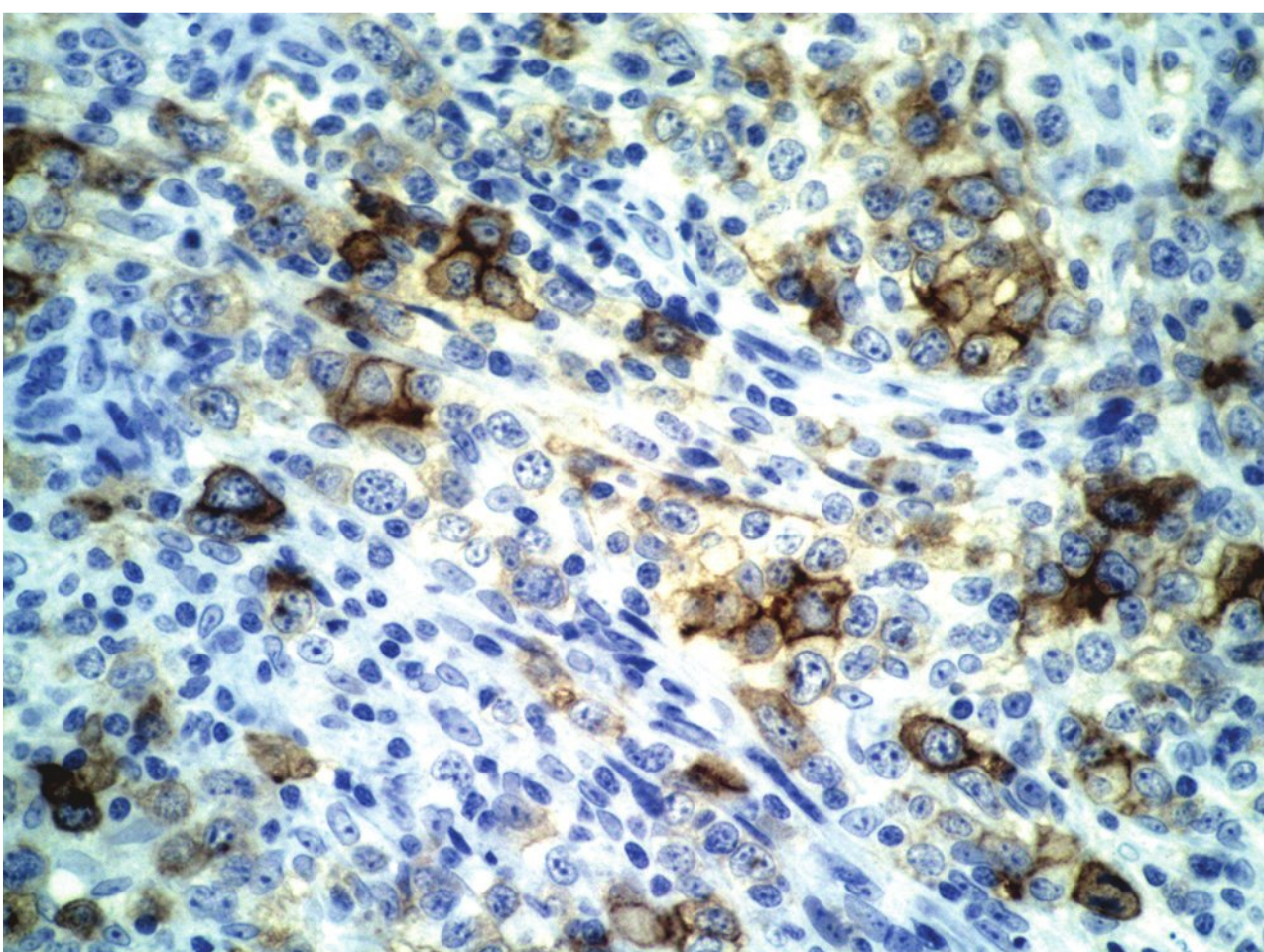


FIGURE 6.44.10 CD30 stain demonstrates scattered immunoblasts. Immunoperoxidase, $\times 40$.

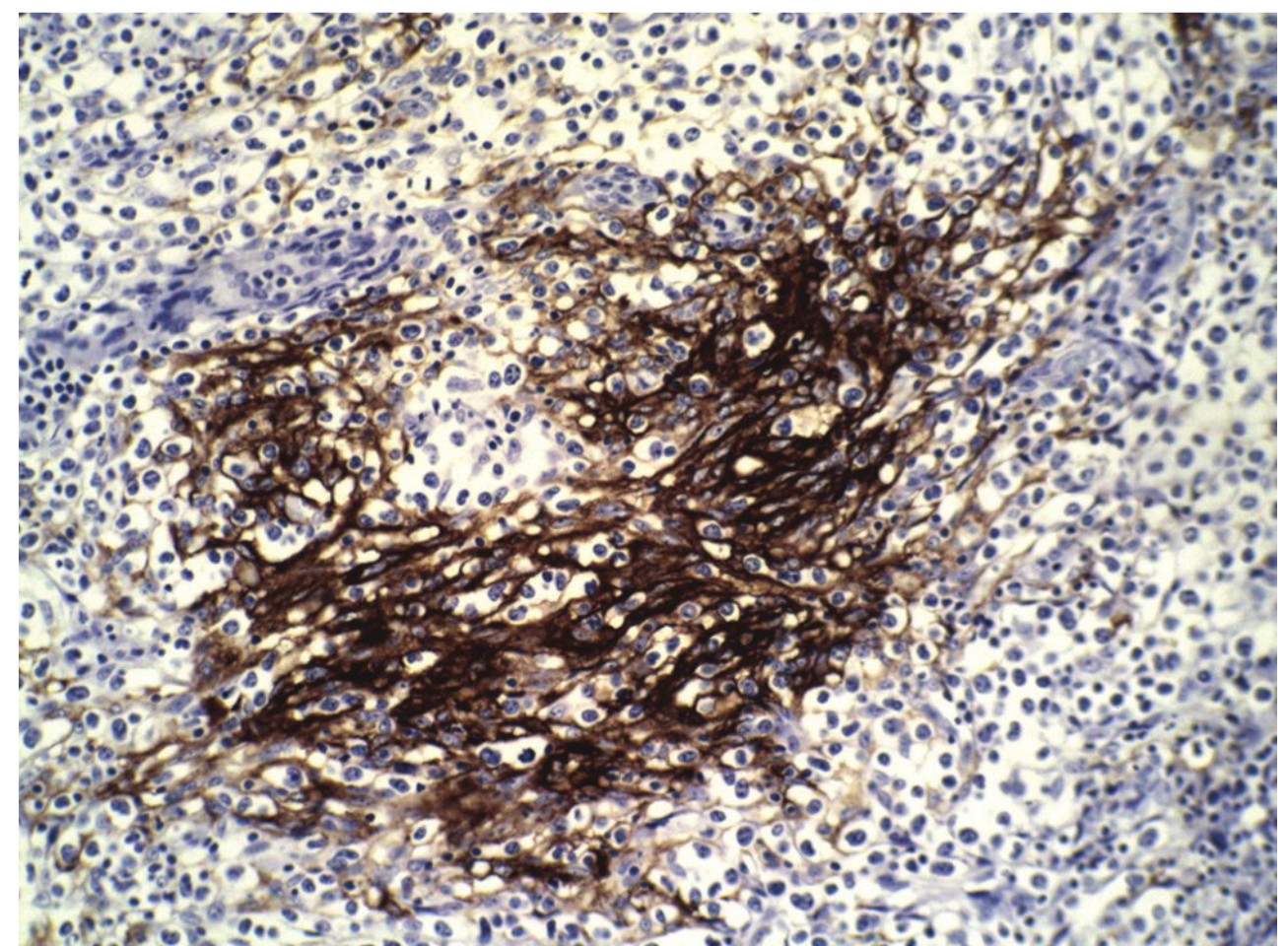


FIGURE 6.44.12 A follicular dendritic meshwork is demonstrated by CD21 staining. Immunoperoxidase, $\times 20$.

TABLE 6.44.1

Morphologic Diagnostic Criteria for AITCL

1. Partial or total effacement of normal lymph node architecture.
2. Paracortical infiltration by polymorphic cellular population evolving into predominantly lymphoma cell population.
3. T-lymphoma cells are of medium- to large-size with clear cytoplasm.
4. Proliferation of arborizing high endothelial venules.
5. Proliferation of extrafollicular follicular dendritic cells.
6. Presence of immunoblasts with EBV infection.

such as follicular hyperplasia in early stages, and immunoblastic and plasmacytic expansion with hypergammaglobulinemia in later stages of AITCL (5,8,12,16). It also induces proliferation of FDCs, one of the characteristic features of AITCL. ICOS is a CD26 homologue implicated in the regulation of T-cell differentiation, and it may be dysregulated in AITCL (16).

Other helpful markers are for the demonstration of FDCs by CD21, CD23, or CD35, detection of B-cell proliferation by B-cell markers (CD20 and CD79A), and staining of EBV by EBV latent membrane protein or in situ hybridization for EBV-encoded RNA (EBER). EBV infection of the B lymphocytes is a constant feature in AITCL (5). It is controversial whether immune deregulation in AITCL induces EBV infection or EBV initiates tumorigenesis (1,8). However, it seems that the pathogenesis of AITCL involves the interactions between EBV, T cells, and B cells.

The current case has clinical symptoms of skin rash, weight loss, and generalized lymphadenopathy. Histologically, there is prominent vascular arborization, proliferation of extrafollicular FDCs, and presence of sheets of clear cytoplasm tumor cells. The tumor cells show a characteristic immunophenotype of follicular helper T cell, as manifested by positive reactions to CD3, CD4, CD10, and BCL-6. Therefore, it is a typical case of AITCL.

Comparison between Flow Cytometry and Immunohistochemistry

Flow cytometric results can be misleading to the interpretation of a reactive process because of the mixed cellular composition in AITCL (6). Immunohistochemistry with a CD3, CD4, CD8, CD10, and BCL-6 panel is specific enough to distinguish AITCL from other T-cell lymphomas. The ability to demonstrate an extrafollicular follicular dendritic meshwork with CD21, CD23, or CD35 staining is also important for the diagnosis. Identification of EBV, though not absolutely necessary, is also supportive for a diagnosis of AITCL.

Molecular Genetics

Almost all cases of AITCL reveal a monoclonal or an oligoclonal rearrangement of TCRb and/or TCRg genes (19). However, monoclonal immunoglobulin heavy-chain gene

TABLE 6.44.2

Salient Features for Laboratory Diagnosis of AITCL

1. Immunophenotype: Follicular helper T cell (T_{FH}) with positive staining for CD3, CD4, CD10, and BCL-6.
2. Specific markers for T_{FH} include CXCL13, PD1, and ICOS.
3. CD21, CD23, or CD35 stain to demonstrate extrafollicular follicular dendritic meshwork.
4. EBV latent membrane protein stain or EBER to demonstrate EBV infection of B cells
5. Clonal TCRb or TCRg rearrangement
6. Clonal immunoglobulin heavy-chain gene rearrangement in cases with immunoblastic proliferation or with transformation to diffuse large B-cell lymphoma
7. GEP demonstrates a T_{FH} pattern

rearrangement has also been identified in a minority of cases (about 25% to 30%) (5). This clonal B-cell population is considered to represent proliferation of the EBV+ B immunoblasts. In a few cases, this clonal population may finally develop into a full-blown feature of a diffuse large B-cell lymphoma (9).

Gene expression profiling (GEP) studies demonstrated overexpression of several genes in AITCL cases, including CXCL13, BCL-6, PDCD1, CD40L, and NFATC1, which are characteristic of normal T_{FH} cells and distinguish AITCL from other peripheral T-cell lymphomas (17,18). In addition, GEP also revealed a strong microenvironment imprint with overexpression of B cell and FDC-related genes, chemokines, and genes related to extracellular matrix and vascular biology.

Chromosomal abnormalities have been detected in the majority of AITCL cases: 70% with conventional cytogenetics and 90% with fluorescence in situ hybridization (1,7,20). The most common cytogenetic aberrations in AITCL include trisomy 3, trisomy 5, and an additional X chromosome (7,20).

The salient features for laboratory diagnosis of AITCL are summarized in Table 6.44.2.

Clinical Manifestations

AITCL is usually seen in elderly patients in the sixth to seventh decades with no sex predilection (1,5–8). Most patients present with a systemic disease with B-symptoms, generalized lymphadenopathy, and hepatosplenomegaly. The majority of patients have skin rash or pruritus. About one quarter of patients may have edema, pleural effusion, and ascites. Autoimmune phenomena are also common features that include autoimmune hemolytic anemia, vasculitis, polyarthritis, rheumatoid arthritis, and autoimmune thyroid disease. Accordingly, patients may have a positive Coomb test, cold agglutinins, cryoglobulins, and circulating immune

complexes. Other common laboratory findings include hypergammaglobulinemia, and elevation of lactate dehydrogenase and erythrocyte sedimentation rate. Many patients also have leukocytosis with neutrophilia or eosinophilia and lymphopenia. The clinical outcome of AITCL patients is dismal with a median survival of <3 years. Most patients may achieve a complete remission, but relapse is common. The cause of death is usually due to spreading of lymphoma or secondary bacterial, viral, or fungal infections.

REFERENCES

1. Dogan A. Angioimmunoblastic T-cell lymphoma. *Br J Haematol*. 2003;121:681–691.
2. Prizzera G, Moran E, Rappaport H. Angio-immunoblastic lymphadenopathy with dysproteinemia. *Lancet*. 1974;1:1070–1073.
3. Lukes R, Tindle B. Immunoblastic lymphadenopathy. A hyperimmune entity resembling Hodgkin's disease. *N Engl J Med*. 1975;292:1–8.
4. Lennert K, Feller AC. Histopathology of Non-Hodgkin's Lymphomas (based on the updated Kiel classification). Berlin, Germany: Springer-Verlag; 1992.
5. Dogan A, Gauland P, Jaffe ES, et al. Angioimmunoblastic T-cell lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al. eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:309–311.
6. Ferry JA. Angioimmunoblastic T-cell lymphoma. *Adv Anat Pathol*. 2002;9:273–279.
7. Iannitto E, Ferreri AJM, Minardi V, et al. Angioimmunoblastic T-cell lymphoma. *Crit Rev Oncol Hematol*. 2008;68:264–271.
8. Dunleavy K, Wilson WH, Jaffe ES. Angioimmunoblastic T-cell lymphoma: pathobiological insights and clinical implications. *Curr Opin Hematol*. 2007;14:348–353.
9. Attygalle AD, Kyriakou C, Dupuis J, et al. Histologic evolution of angioimmunoblastic T-cell lymphoma in consecutive biopsies, clinical consultation and insights into natural history and disease progression. *Am J Surg Pathol*. 2007;31:1077–1088.
10. Attygalle A, Al-Jehani R, Diss TC, et al. Neoplastic T-cells in angioimmunoblastic T-cell lymphoma express CD10. *Blood*. 2002;99:627–633.
11. Cho YU, Chi HS, Park CJ, et al. Distinct features of angioimmunoblastic T-cell lymphoma with bone marrow involvement. *Am J Clin Pathol*. 2009;131:640–646.
12. Khokhar FA, Payne WD, Talwalkar SS, et al. Angioimmunoblastic T-cell lymphoma in bone marrow: a morphologic and immunophenotypic study. *Hum Pathol*. 2010;41(1):79–87.
13. Dogan A, Morice WG. Bone marrow histopathology in peripheral T-cell lymphomas. *Br J Haematol*. 2004;127:140–154.
14. Grogg KL, Morice WG, Macon WR. Spectrum of bone marrow findings in patients with angioimmunoblastic T-cell lymphoma. *Br J Haematol*. 2007;137:416–422.
15. Martel P, Laroche L, Courville P, et al. Cutaneous involvement in patients with angioimmunoblastic lymphadenopathy with dysproteinemia. *Arch Dermatol*. 2000;136:881–886.
16. Rodriguez-Justo M, Attygalle AD, Munson P, et al. Angioimmunoblastic T-cell lymphoma with hyperplastic germinal centres: a neoplasia with origin in the outer zone of the germinal centre? Clinicopathological and immunohistochemical study of 10 cases with follicular T-cell markers. *Mod Pathol*. 2009;22:753–761.
17. de Leval L, Richman DS, Thielen C, et al. The gene expression profile of nodal peripheral T-cell lymphoma demonstrates a molecular link between angioimmunoblastic T-cell lymphoma and follicular helper T-cells. *Blood*. 2007;109:4952–4963.
18. Piccaluga PP, Agostinelli C, Califano A, et al. Gene expression analysis of angioimmunoblastic T-cell lymphoma indicates derivation from T-follicular helper cells and vascular endothelial growth factor deregulation. *Cancer Res*. 2007;67:10703–10710.
19. Shah ZH, Harris S, Smith JL, et al. Monoclonality and oligoclonality of T cell receptor b gene in angioimmunoblastic T cell lymphoma. *J Clin Pathol*. 2009;62:177–181.
20. Schlegelberger B, Zwingers T, Hohenadel K, et al. Significance of cytogenetic findings for the clinical outcome in patients with T-cell lymphoma of angioimmunoblastic lymphadenopathy type. *J Clin Oncol*. 1996;14:593–599.

CASE 45

Anaplastic Large-Cell Lymphoma

CASE HISTORY

A 14-year-old boy with fever and sore throat for a week was admitted to the hospital for evaluation. The patient was treated with antibiotics before admission but showed no response. His mother claimed that the patient had apparent weight loss in the last 2 weeks. Physical examination on admission showed lymphadenopathy involving the cervical and axillary regions. His sedimentation rate was

elevated. A monospot test was negative. Serologic examination for antibodies against toxoplasma, cytomegalovirus, and Herpes simplex was negative. Peripheral blood examination showed mild lymphocytosis, but the morphology of the lymphocytes was normal. His hemoglobin was 12 g/dL, and hematocrit 36%. The platelet count was 190,000/mL. A lymph node biopsy was performed and revealed morphologic evidence of lymphoma. A subsequent bone marrow biopsy also showed tumor involvement.

FLOW CYTOMETRY FINDINGS

Lymph node biopsy: B-cell markers: CD10 0%, CD19 10%, CD20 4%, CD23 1%, k 6%, l 4%. T-cell markers: CD3/CD4 22%, CD3/CD8 72%, CD5 69%, CD7 9%. Monocyte marker: CD14 2%. Activation antigen: CD30 76%.

Bone marrow aspirate: B-cell markers: CD19 12%, CD20 6%, CD23 0%, k 8%, l 4%. T-cell markers: CD3/CD4 16%, CD3/CD8 64%, CD5 58%, CD7 7%. Monocyte marker: CD14 1%. Activation antigen: CD30 96% (Fig. 6.45.1).

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining showed that the tumor cells were positive for CD30, epithelial membrane antigen (EMA), anaplastic lymphoma kinase 1 (ALK1), CD3, and CD8, but negative for CD15 and CD20.

MOLECULAR GENETIC FINDINGS

Cytogenetic karyotyping revealed t(2;5)(p23;q35) translocation.

DISCUSSION

In the search of Reed-Sternberg cell-specific antigens, a new antigen, designated Ki-1 antigen, was discovered in 1982 and was later clustered as CD30. Subsequently, CD30 was identified in a group of diffuse large-cell lymphomas; this group of tumors was then called Ki-1+ anaplastic large-cell lymphoma (ALCL) or CD30+ ALCL (1). However, because CD30 is also expressed in other tumors, including Hodgkin lymphoma, embryonal carcinoma, body cavity lymphoma, mediastinal B-cell lymphoma, and cutaneous T-cell lymphoma (2), the descriptive term Ki-1 or CD30 was dropped from the designation. This group of tumors is now called ALCL in the Revised European-American classification of lymphoid neoplasms (REAL) and World Health Organization (WHO) classification (3,4). In the 2008 WHO classification, ALCL is further divided into ALCL, ALK-positive, and ALCL, ALK-negative subtypes (5,6). The ALCL, ALK- subtype is considered a provisional entity with similar morphology and immunophenotype as ALCL, ALK+, except for the absence of ALK protein (5,6).

Morphology

In recent years, several variants of ALCL have been identified. In early reports, ALCL was divided into several types:

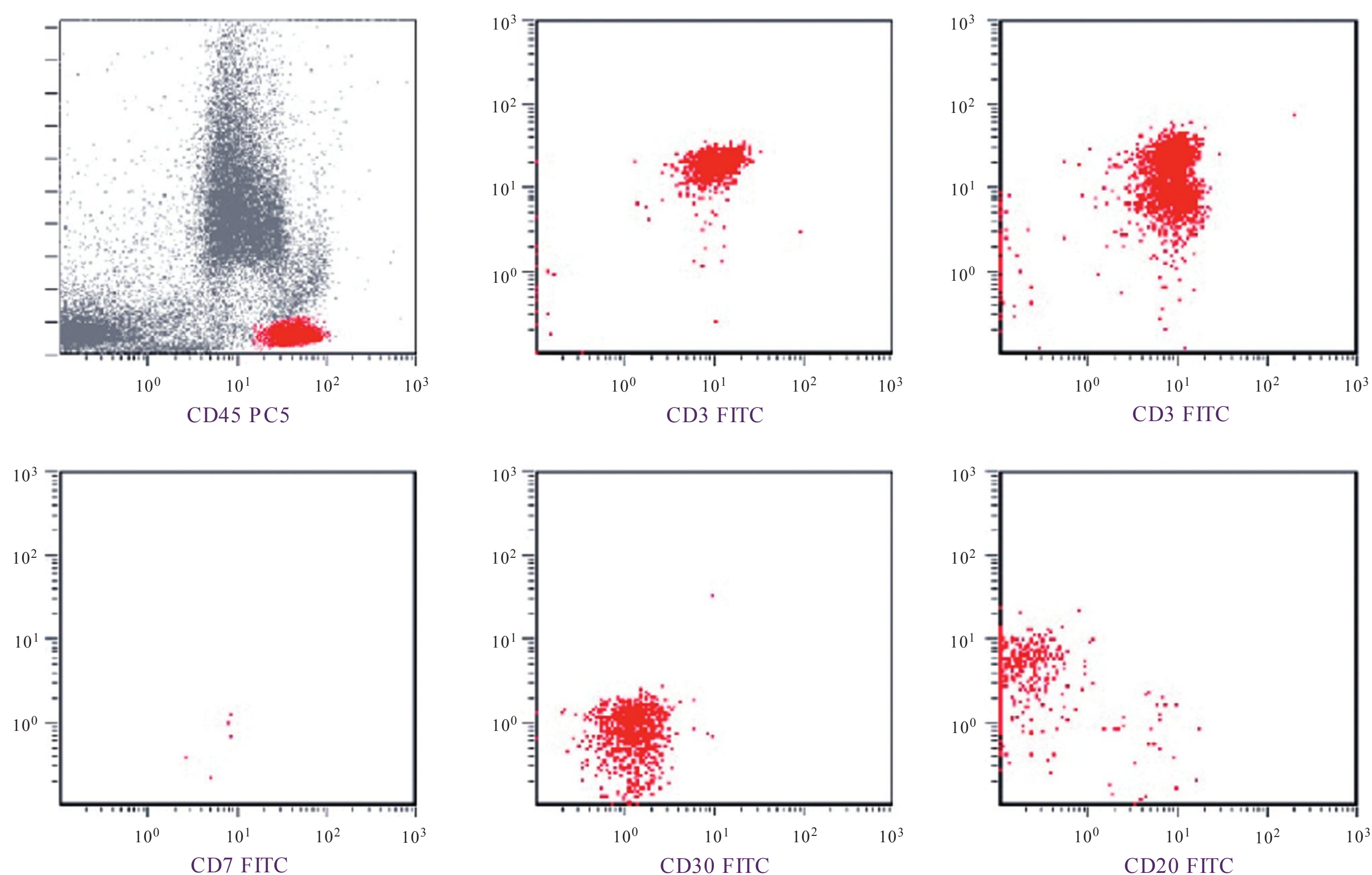


FIGURE 6.45.1 Flow cytometric analysis of bone marrow shows the presence of CD3-/CD4-, CD3-/CD8-, CD5-, and CD30-positive cells but absence of CD7- and CD20-positive cells. SS, side scatter; PC5, phycoerythrin-cyanin 5; RD1, rhodamine; FITC, fluorescein isothiocyanate; PE, phycoerythrin.

pleomorphic (common or classic), monomorphic, small cell, lymphohistiocytic, Hodgkin-like, and mixed (7–12). In addition, there are reported cases that show large numbers of fibroblastlike spindle cells (sarcomatoid variant) (13), neutrophils (neutrophil-rich) (14), eosinophils (eosinophil-rich) (15), and signet-ring cells (signet-ring appearance) (16), but these rare morphologic forms are not considered to be distinct subtypes by most authors. In the old WHO classification, there are only three variants: (a) common, (b) small cell, and (c) lymphohistiocytic (7). In the 2008 WHO classification, the term “variant” is replaced by “pattern,” and a Hodgkin-like pattern is added (5,6).

The common pattern consists of a wide spectrum of large tumor cells with different morphologic features and was originally designated the polymorphic variant. It accounts for 60% of ALCL cases (5,6). The most common cell type has chromatin-poor horseshoe- or kidney-shaped nuclei with multiple nucleoli (Fig. 6.45.2). Ring-shaped nuclei (doughnut cell) can also be seen. Some authors emphasize the presence of a perinuclear eosinophilic region, which probably represents a prominent Golgi apparatus (11). These tumor cells have a moderate amount of mildly basophilic cytoplasm, which may contain vacuoles that are only visible in touch preparations (8,9). Cells with these features have been called hallmark cells because they are present in all ALCL patterns in various proportions, and their presence is the major diagnostic criterion.

Multinucleated giant tumor cells are frequently demonstrated in the common pattern; when giant cells are predominant, it was designated the giant cell-rich variant (12). However, this designation is no longer recognized in the new classification. The monomorphic variant, which is a rare morphologic manifestation, is also currently included in the common pattern (8,9).

The Hodgkin-like pattern contains a nodular sclerosis pattern and large numbers of packed or dispersed tumor cells with features resembling those of classic Hodgkin and

Reed-Sternberg cells (Fig. 6.45.3). However, the current thinking is that many cases reported with this pattern may represent a tumor cell-rich variant of classic Hodgkin lymphoma and not a true ALCL (8,9). Nevertheless, the 2008 WHO classification includes this pattern as a rare entity (3%) (5,6).

The small-cell pattern, accounting for 5% to 10% of ALCL cases is characterized by the presence of predominantly small-to-medium-sized tumor cells (17) (Fig. 6.45.4). Therefore, it is frequently misdiagnosed as peripheral T-cell lymphoma, unspecified, if CD30 and other special stains are not performed (7). However, the hallmark cells are invariably identifiable around the blood vessels, particularly with the help of CD30 staining.

The lymphohistiocytic pattern, accounting for 10% of ALCL cases, is defined by the presence of a large number of histiocytes, which may occasionally show signs of erythrophagocytosis (Fig. 6.45.5) (18). As in the small-cell pattern, a diagnosis depends on the finding of perivascular infiltration of the large tumor cells.

In approximately 10% of cases, more than one variant can be present in the same patient (7,19). In addition, in case of relapses, a variant different from that seen in the initial diagnosis may appear (7,19).

The general histologic features are characterized by a sheetlike cohesive growth pattern with the tendency of sinusoidal infiltration (Fig. 6.45.6), a phenomenon mimicking carcinoma. When the lymph node is not completely obliterated, a perifollicular pattern may be demonstrated. As mentioned before, another special feature is the perivascular infiltration by the large tumor cells, which is especially striking in the small-cell and lymphohistiocytic variants.

The tumor cell morphology of ALCL, ALK[−] subtype is similar to ALCL, ALK⁺, but a small-cell pattern is not recognized in the former (5,6). Tumor cells in ALCL, ALK[−] subtype tend to be larger and more pleomorphic than those seen in ALCL, ALK⁺ subtype and have a higher nuclear-cytoplasmic ratio. (5,6).

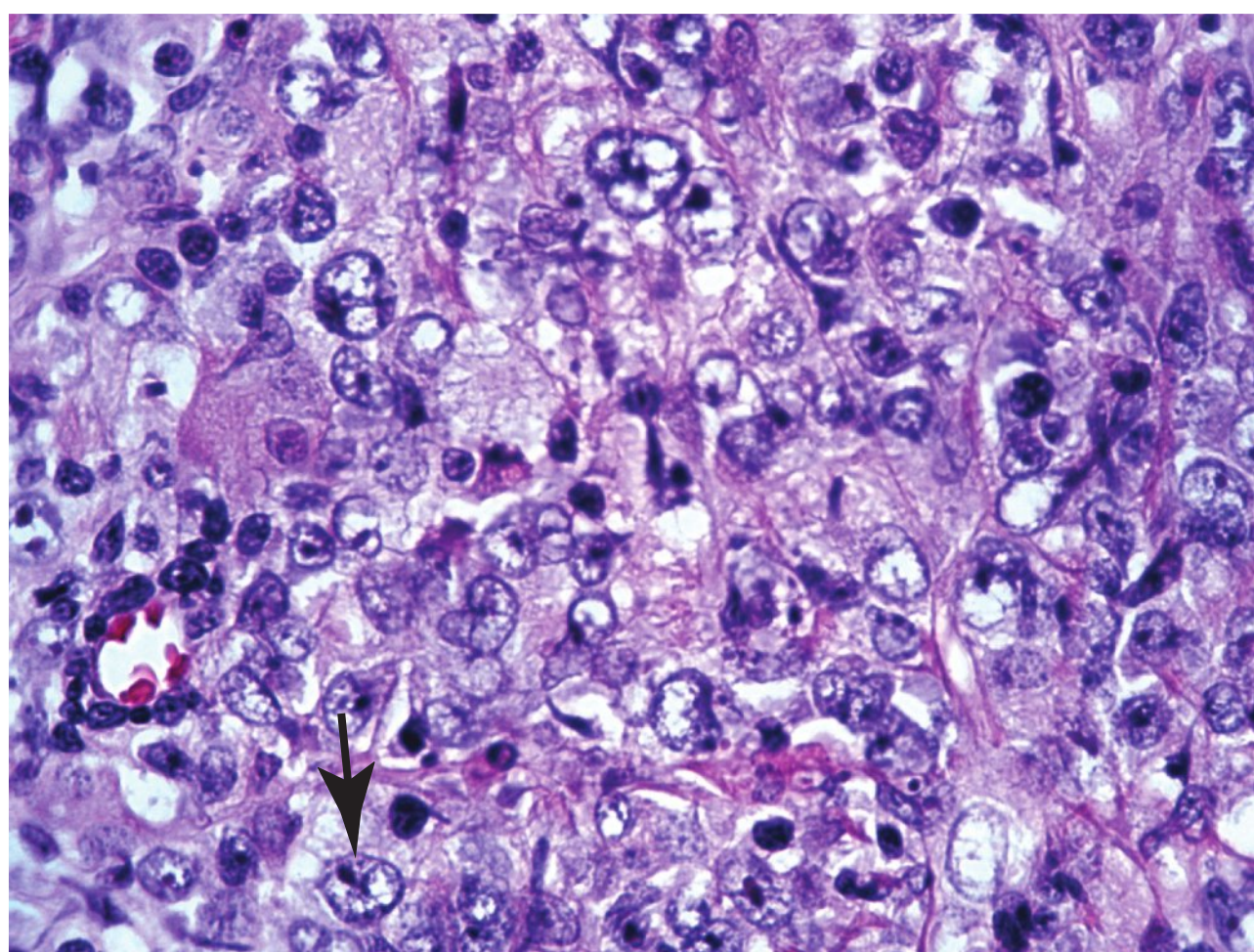


FIGURE 6.45.2 Case of the common pattern of ALCL shows cohesive clusters of large tumor cells with chromatin-poor nuclei and prominent nucleoli. Some tumor cells reveal the characteristic kidney-shaped nucleus (arrow). Hematoxylin and eosin, 40× magnification.

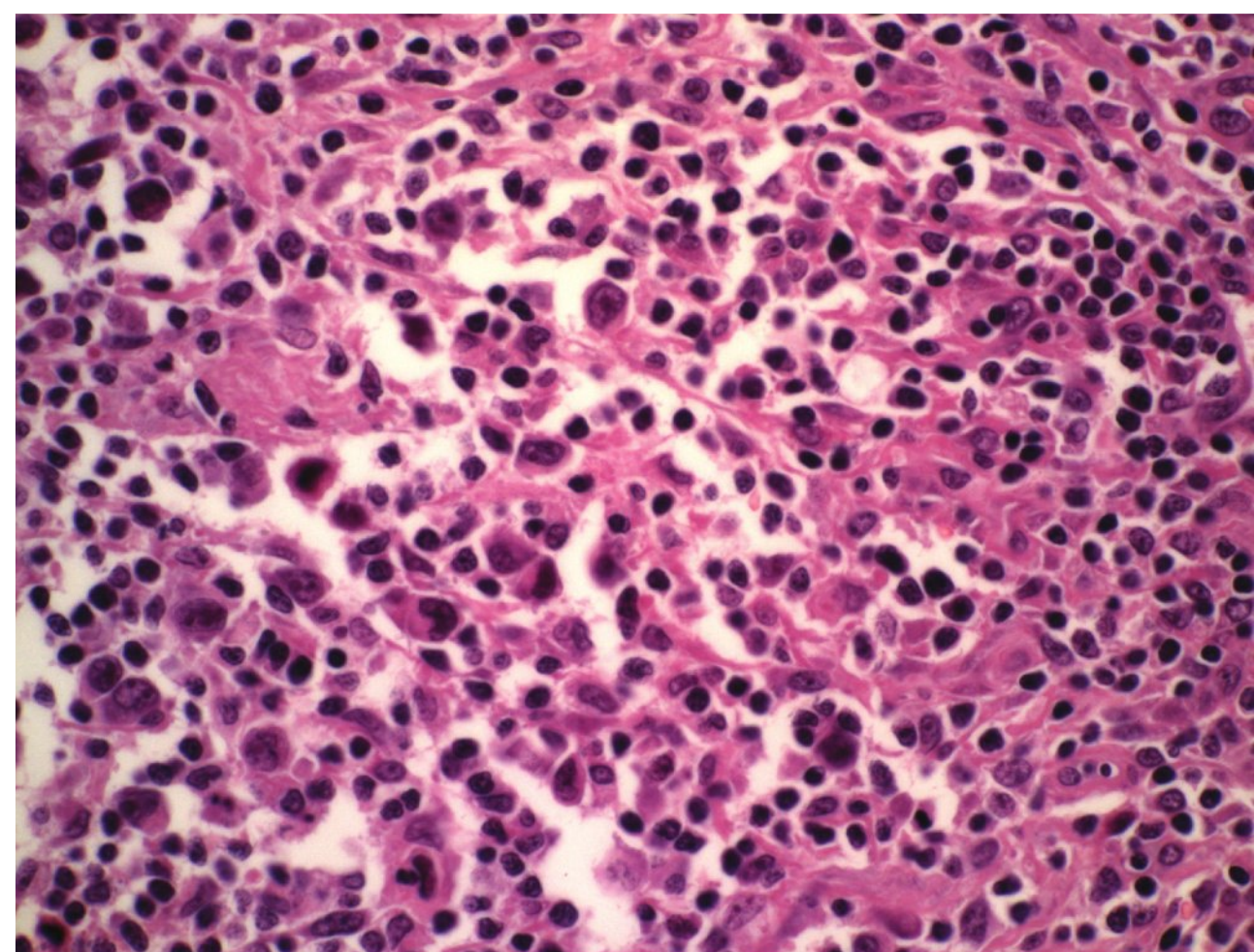


FIGURE 6.45.3 Case of the Hodgkin-like pattern reveals scattered large tumor cells on a background of non-neoplastic small lymphocytes, mimicking Hodgkin lymphoma. Note a sclerotic band (upper field). Hematoxylin and eosin, 40× magnification.

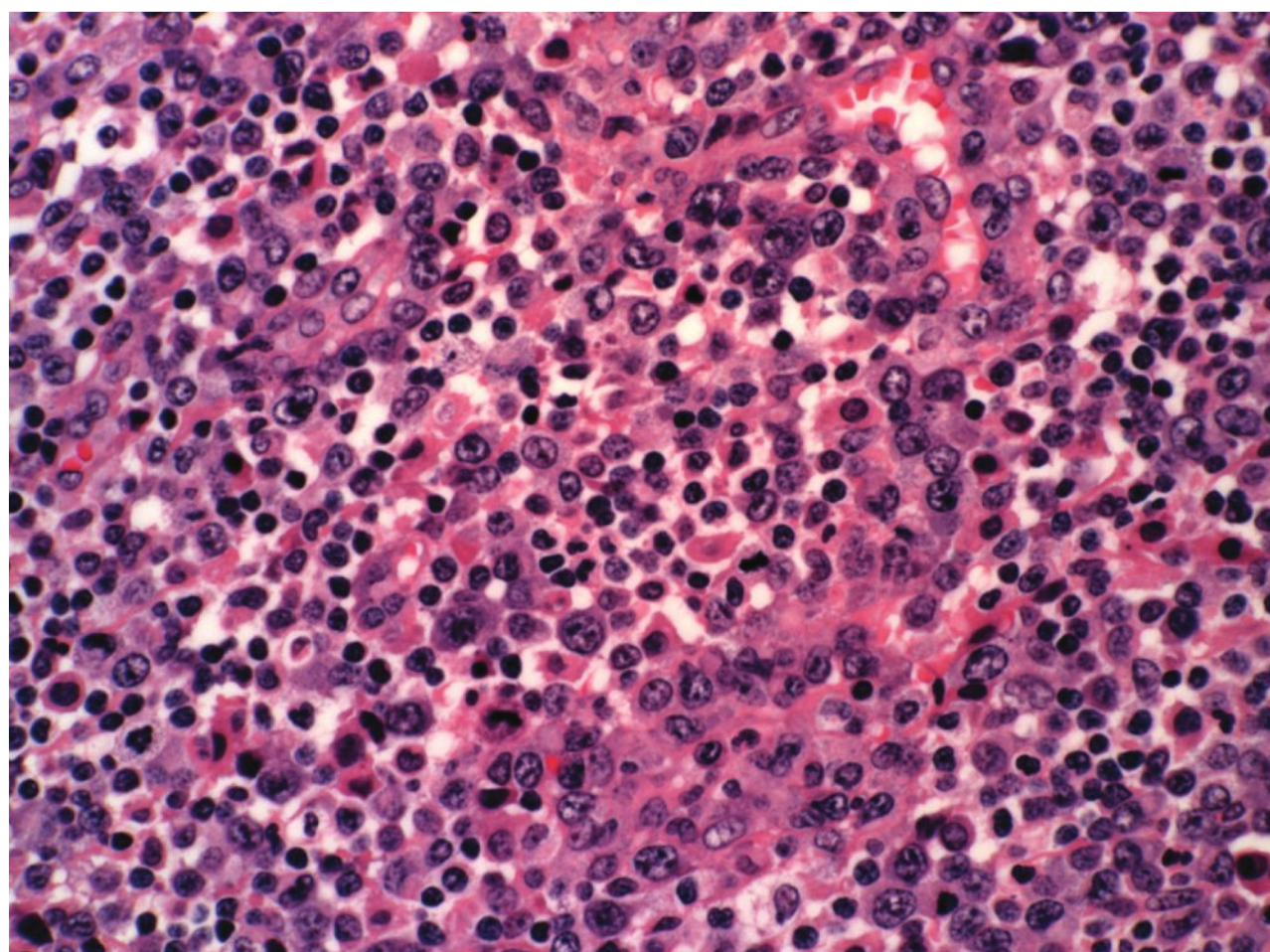


FIGURE 6.45.4 Case of the small-cell pattern shows predominantly small tumor cells. Large tumor cells are concentrated around the blood vessels. Hematoxylin and eosin, 40× magnification.

In primary cutaneous ALCL, the tumor cells are usually large and anaplastic (Fig. 6.45.7). Sheets of tumor cells may infiltrate collagen fibers, skin appendages, and subcutaneous fatty tissue (2). Mitoses and apoptosis are frequent features. The demonstration of vascular invasion may help to distinguish it from lymphomatoid papulosis. The distinction between these two entities is usually difficult morphologically, and observation of the clinical course is usually needed for a definitive diagnosis (19). The so-called neutrophil-rich variant can be seen in cutaneous ALCL in which neutrophilic infiltration may mask the tumor cells (21). A detailed description of primary cutaneous ALCL is presented in Case 46. The characteristic morphologic features of ALCL are summarized in Table 6.45.1.

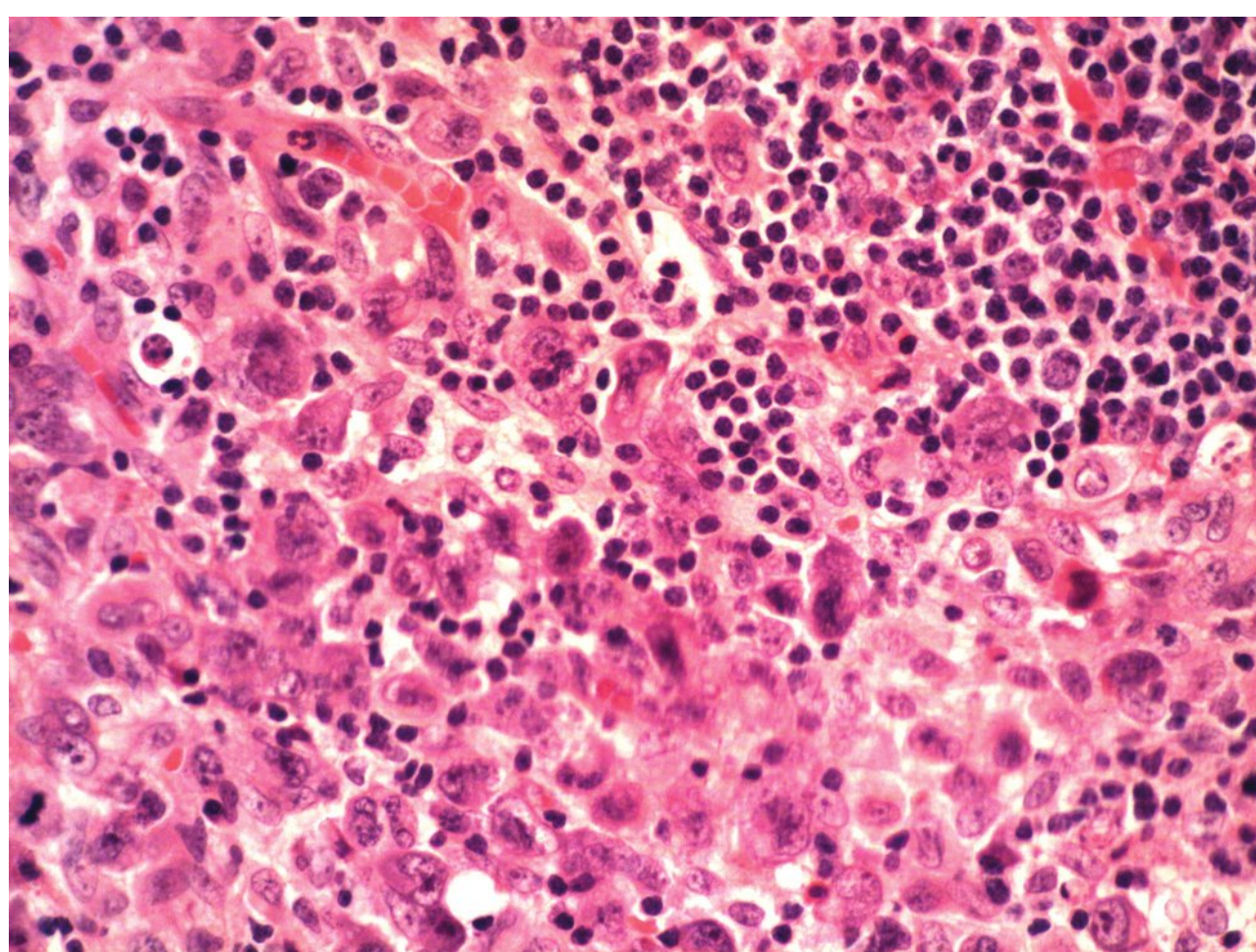


FIGURE 6.45.5 Case of the lymphohistiocytic pattern reveals abundant histiocytes intermixing with ALCL tumor cells. Hematoxylin & Eosin, 40× magnification.

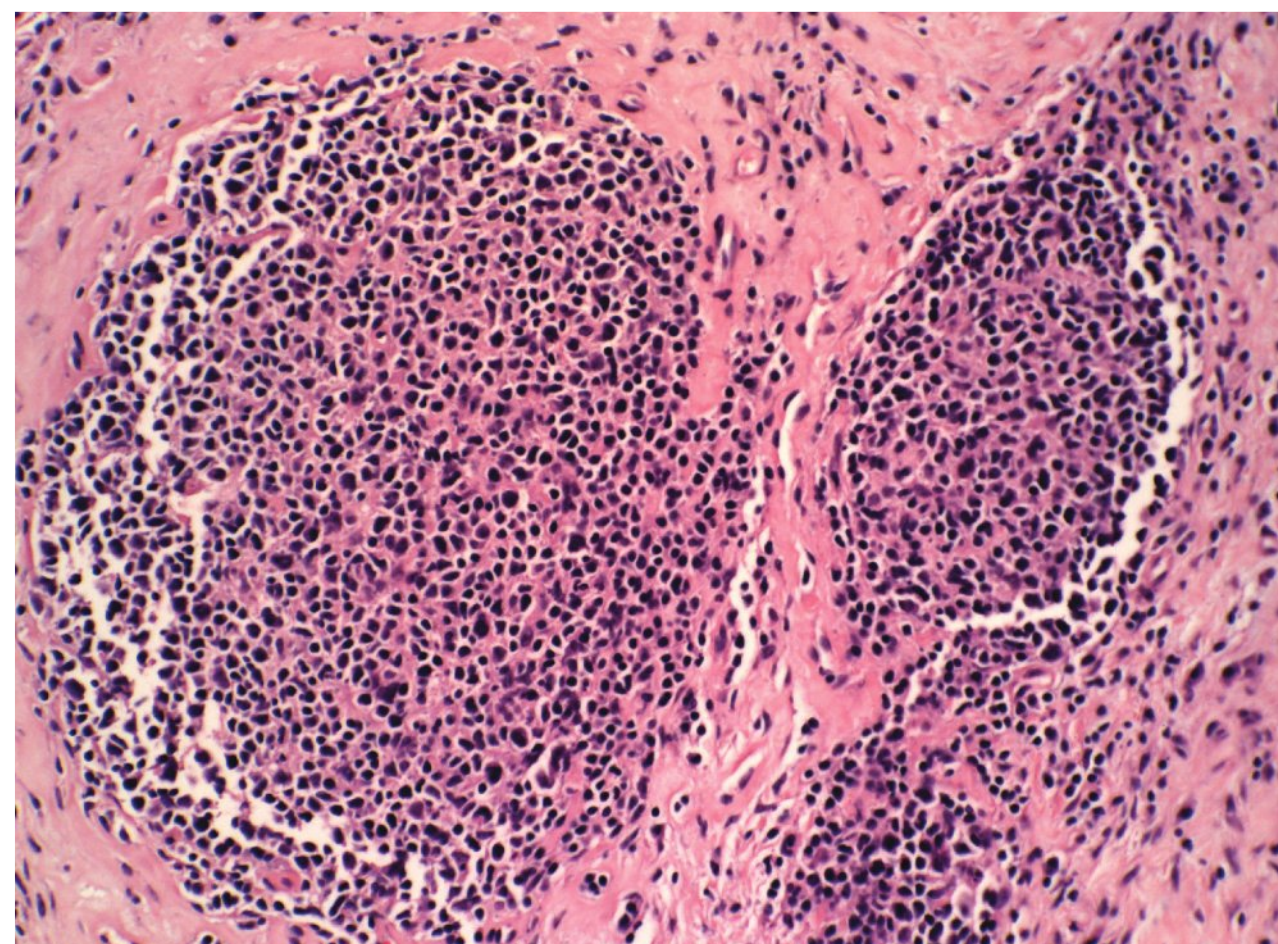


FIGURE 6.45.6 Case of common pattern demonstrates a sinusoidal infiltration pattern. Hematoxylin and eosin, 20× magnification.

Immunophenotype

In the REAL and WHO classifications, only T-cell and null cell tumors with positive CD30 are included as ALCL. In the Kiel classification, a B-cell ALCL is recognized, but the general consensus is that B-cell ALCL is closer to diffuse large B-cell lymphoma than to ALCL because it differs from the T-cell and null cell types in clinical, cytogenetic, and molecular aspects (22). In addition, the B-cell tumor is frequently associated with Epstein–Barr virus, whereas the T- and null cell tumors are not (8,9).

Immunohistochemistry is the mainstay for immunophenotyping of ALCL. There are several important characteristic markers for the diagnosis of ALCL—namely, CD30, ALK, clusterin, and EMA—that can be demonstrated by immunohistochemical staining.

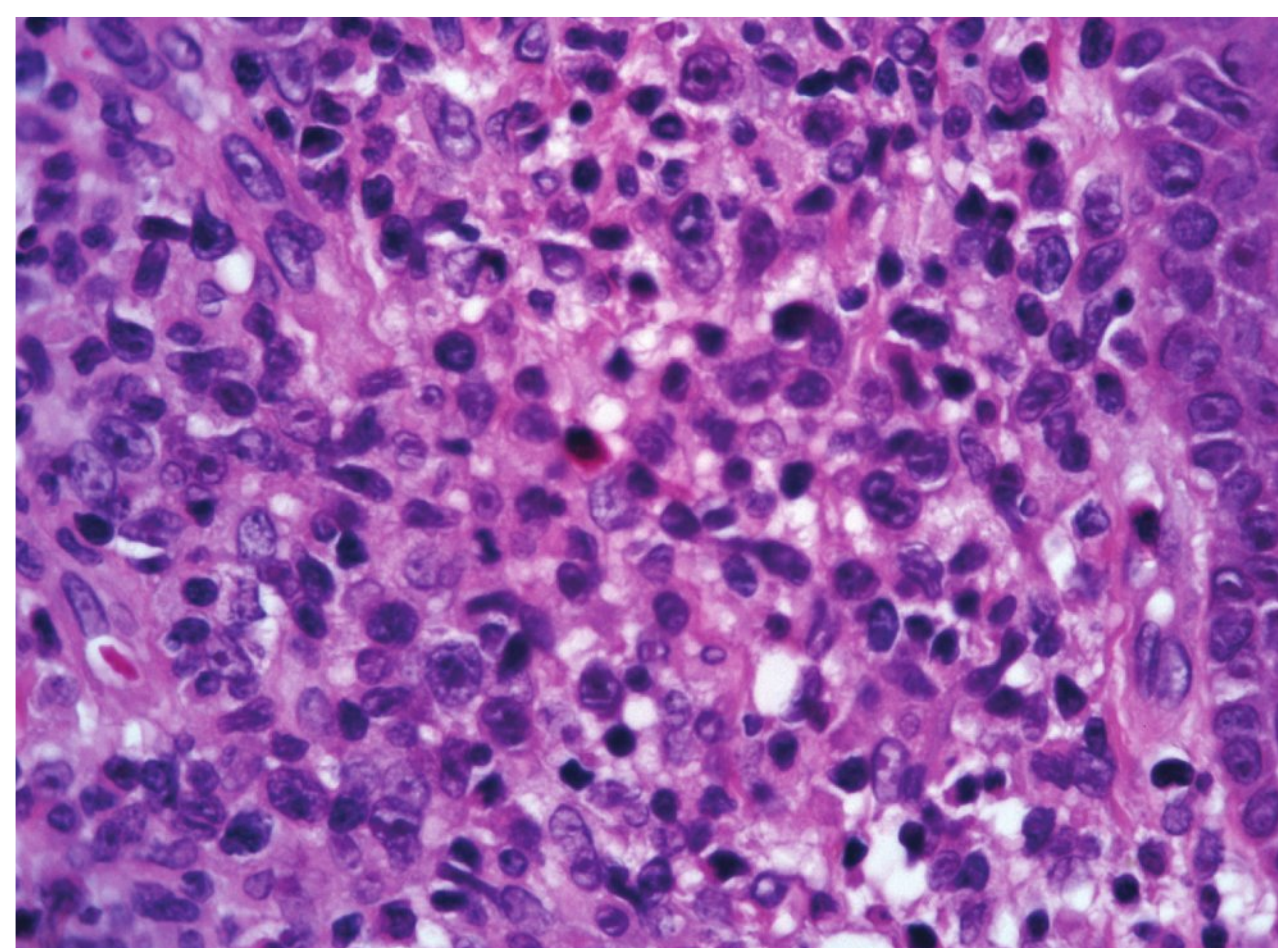


FIGURE 6.45.7 Case of primary cutaneous ALCL shows large tumor cell infiltration intermixing with small lymphocytes. Hematoxylin and eosin, 60× magnification.

TABLE 6.45.1	
Characteristic Morphologic Features of ALCL	
Histologic pattern	Diffuse cohesive proliferation of tumor cells, preferentially infiltrating the sinusoids Perifollicular pattern in early lesions Perivascular large-cell infiltration in small cell and lymphohistiocytic variants
Cytology	Hallmark cells have chromatin-poor horseshoe-shaped nuclei with multiple nucleoli and a perinuclear eosinophilic region. Moderate amount of mildly basophilic cytoplasm that may show vacuoles in touch preparations
Specific features	Demonstration of diffusely infiltrating hallmark cells or perivascular infiltration of anaplastic large cells with admixture of other cell components (small tumor cells, histiocytes, neutrophils, eosinophils)

CD30, by definition, should be present in every case of ALCL. However, the reaction to CD30 staining differs in various cell types (8,9). CD30 stains strongly for the large tumor cells or hallmark cells (Figs. 6.45.8 and 6.45.9). It stains weakly or negatively for small tumor cells in the

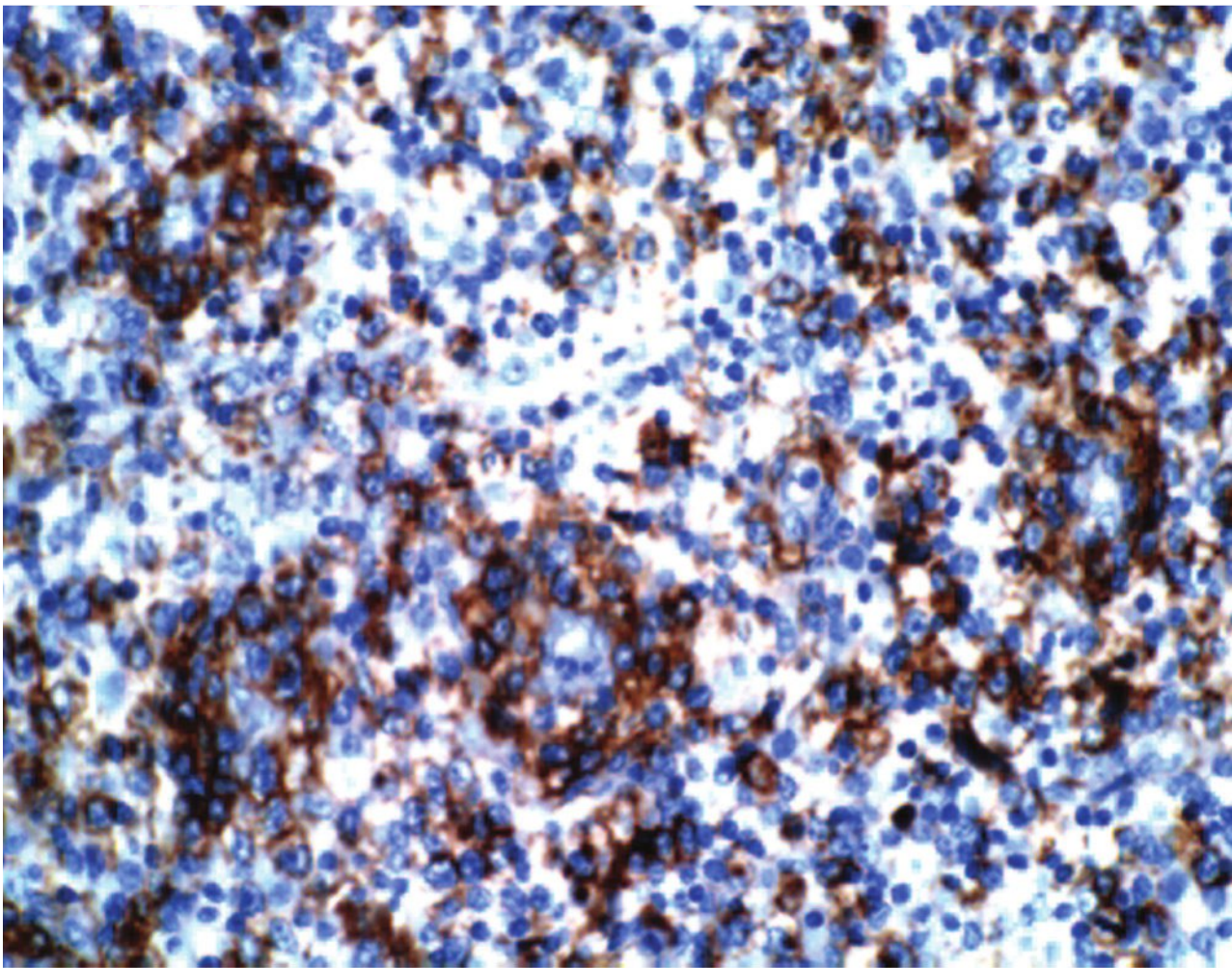


FIGURE 6.45.8 Case of small-cell pattern reveals that CD30 preferentially stains for large tumor cells around the blood vessels. Immunoperoxidase, 40× magnification. (Case provided by Dr. Xiyuan Liang, The Children’s Hospital, Denver, CO.)

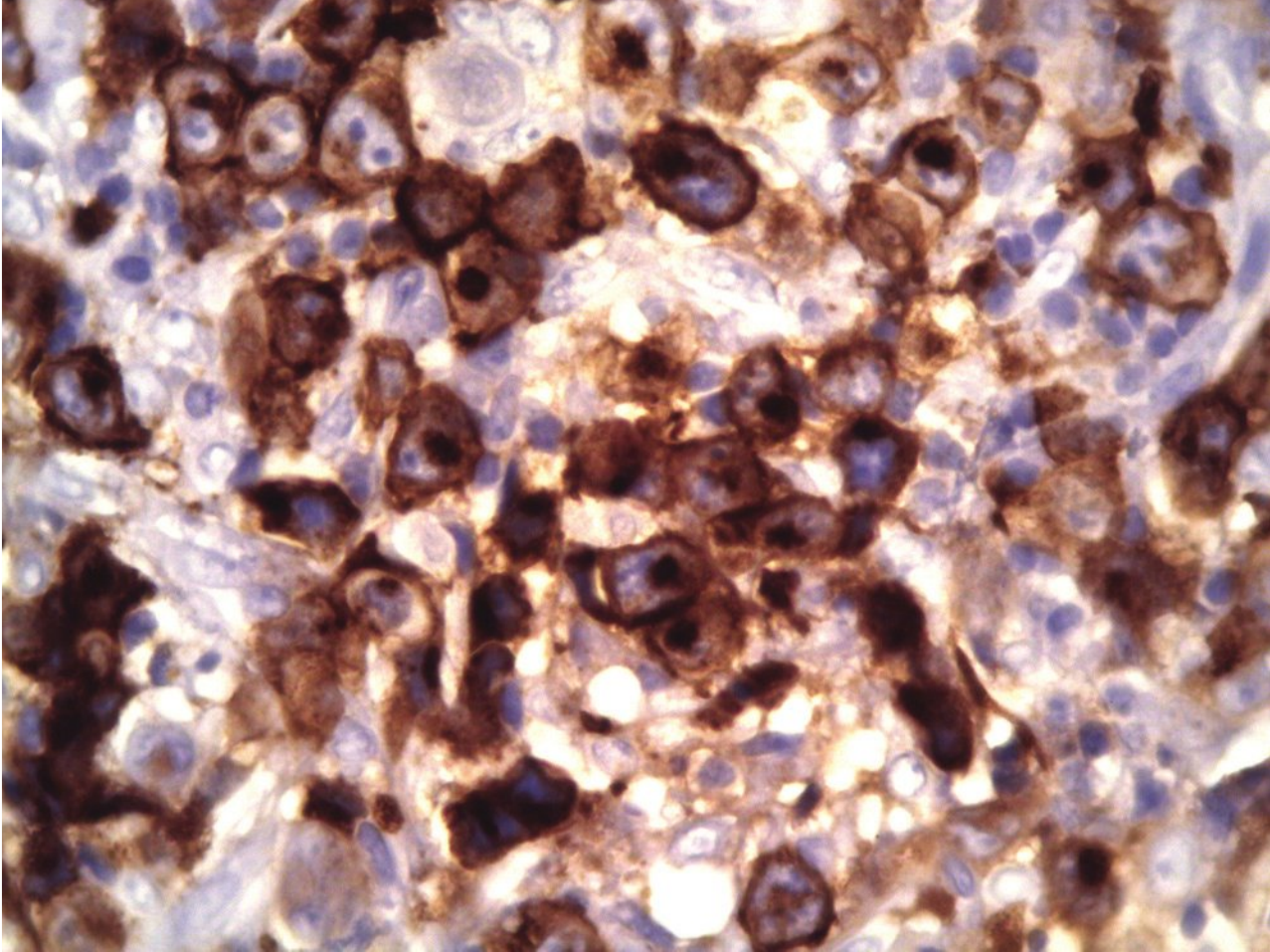


FIGURE 6.45.9 Case of the lymphohistiocytic pattern shows CD30 staining only on large tumor cells. Immunoperoxidase, 60× magnification.

small-cell pattern. The histiocytes in the lymphohistiocytic pattern are negative for CD30 but positive for CD68. The characteristic staining pattern for CD30 is the presence of both membranous and Golgi staining.

The immunohistochemical demonstration of the ALK protein is not only specific for the diagnosis of ALCL but also a reliable prognostic predictor (10,11,23). Therefore, it has been used to divide the clinical cases into ALK-positive and ALK-negative subtypes. The availability of ALK antibodies helps to detect more histologic variants, which could have been misdiagnosed on a morphologic basis (11).

The distribution of ALK differs depending on the karyotypes (2). When the karyotype is t(2;5), which represents ALK/NPM (nucleophosmin) translocation, both nuclear and cytoplasmic staining for ALK occur. Because NPM is a nucleolar shuttling protein, any variant without NPM shows only cytoplasmic staining (2). In terms of cytology, ALK is usually present in both nucleus and cytoplasm of the anaplastic large cells but only in the nucleus of the small tumor cells (Fig. 6.45.10).

Under normal conditions, ALK is only demonstrated in a few scattered cells in the nervous system, including some glial cells, a few endothelial cells, and some pericytes. In diseases, ALK has been reported in rhabdomyosarcoma, inflammatory myofibroblastic tumors, neuroblastomas, and rare large B-cell lymphomas (8).

Clusterin is a relatively new marker for ALCL, but it is highly specific and thus important in differential diagnosis (24). Clusterin has not been demonstrated in Hodgkin lymphoma, but extensive studies have not been done (2,7,20). In the earlier literature, clusterin was reported to be negative in primary cutaneous ALCL, but recent studies have found that the positive rate ranges from 50% to 67% (25).

EMA is considered a marker of carcinoma, and is rarely seen in lymphoma. However, EMA has been demonstrated in most cases of the common pattern, small-cell pattern, and lymphohistiocytic pattern of ALCL (8,9) (Fig. 6.45.11).

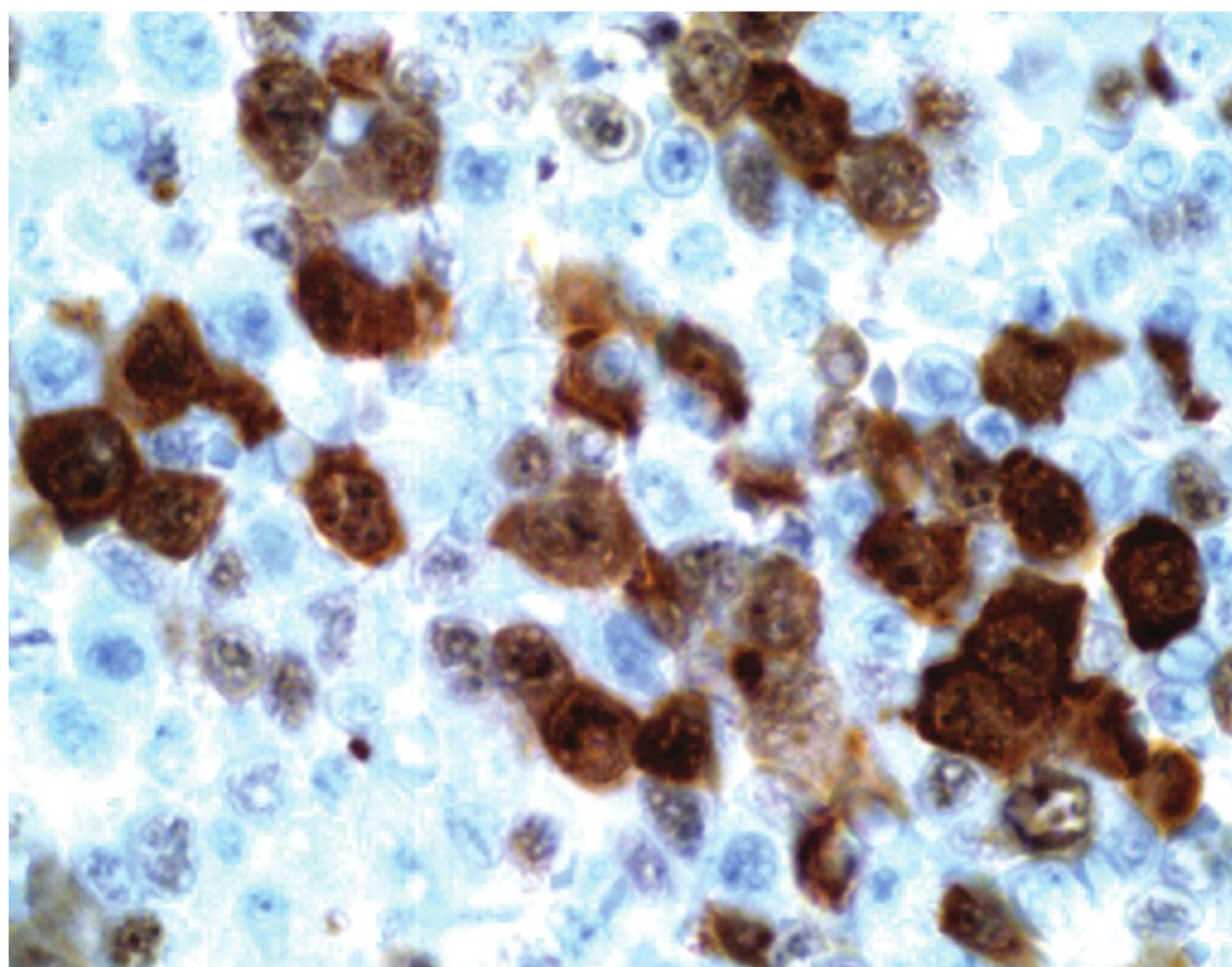


FIGURE 6.45.10 Case of small-cell pattern reveals ALK1 staining of both large and small tumor cells. Large cells stain for both nucleus and cytoplasm, whereas small cells only express weak nuclear staining. Many small cells are negative. Immunoperoxidase, 100× magnification.

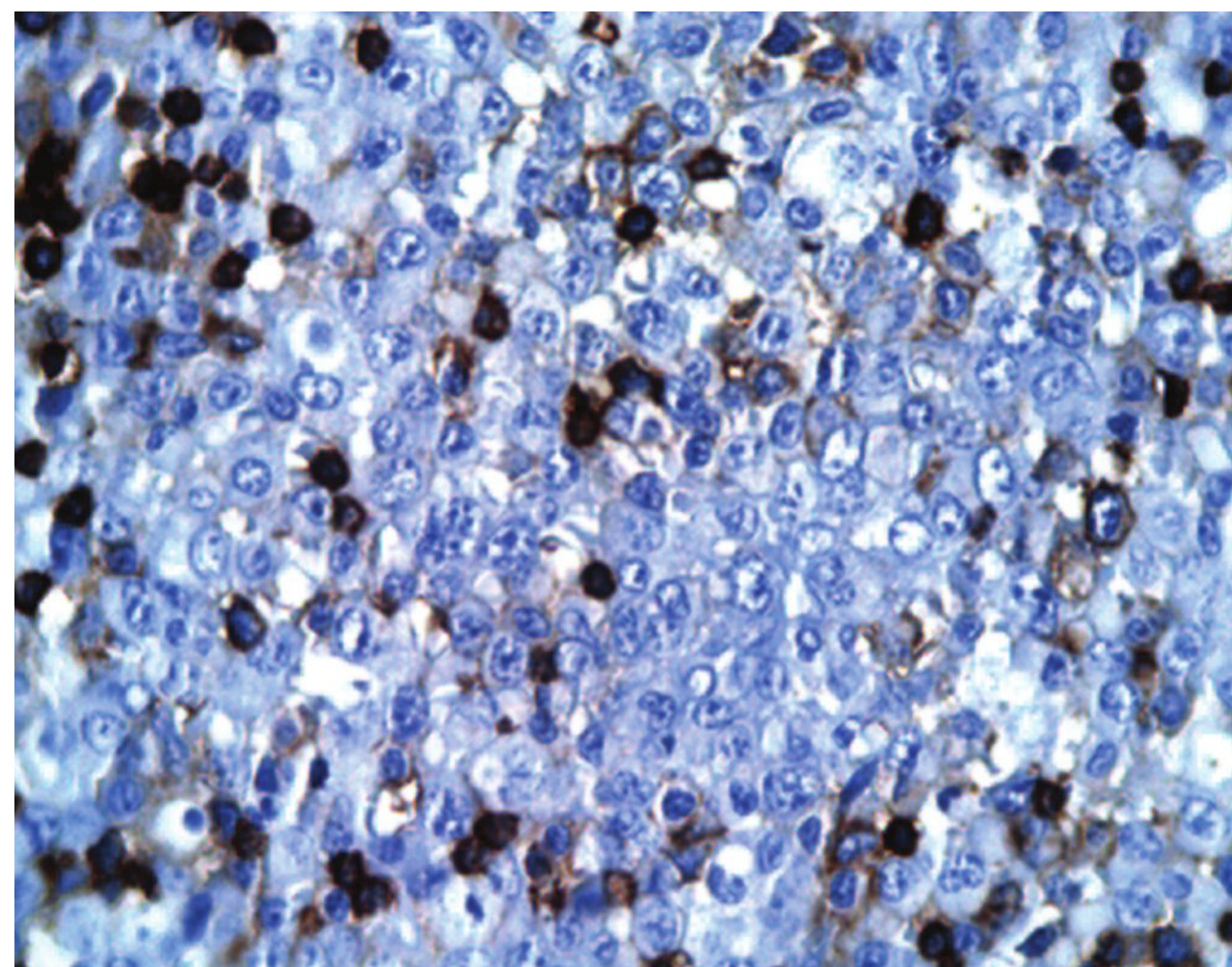


FIGURE 6.45.12 Case of small-cell pattern reveals CD45 staining mainly in small tumor cells. Large tumor cells show negative staining. However, large tumor cells can be positive for CD45. Immunoperoxidase, 60× magnification.

EMA is negative in primary cutaneous ALCL and in Hodgkin lymphoma (2,20). It is present in a minority of ALCL, ALK-cases (5,6). Because one third of ALCL cases may not express CD45 (LCA) (Fig. 6.45.12), the positive EMA reaction may mislead the diagnosis to carcinoma (26).

As mentioned before, ALCL is mainly a T-cell tumor. However, ALCL is characterized by an aberrant T-cell phenotype, lacking one or more pan-T-cell markers (2,11). Therefore, if a large panel of T-cell markers is not used, the tumor may be mislabeled as null cell type. Nevertheless, even in those so-called null cell cases, T-cell lineage can still be identified at the genetic level (5–7). CD3 is most frequently negative, followed by CD5 and CD7 (5–7). CD2 and CD4 are more often positive and should be used for the identification of cell lineage. Other T-cell markers, such as CD43 and CD45RO, are variably positive (5–7). The demonstration of these markers can help rule out the null cell phenotype.

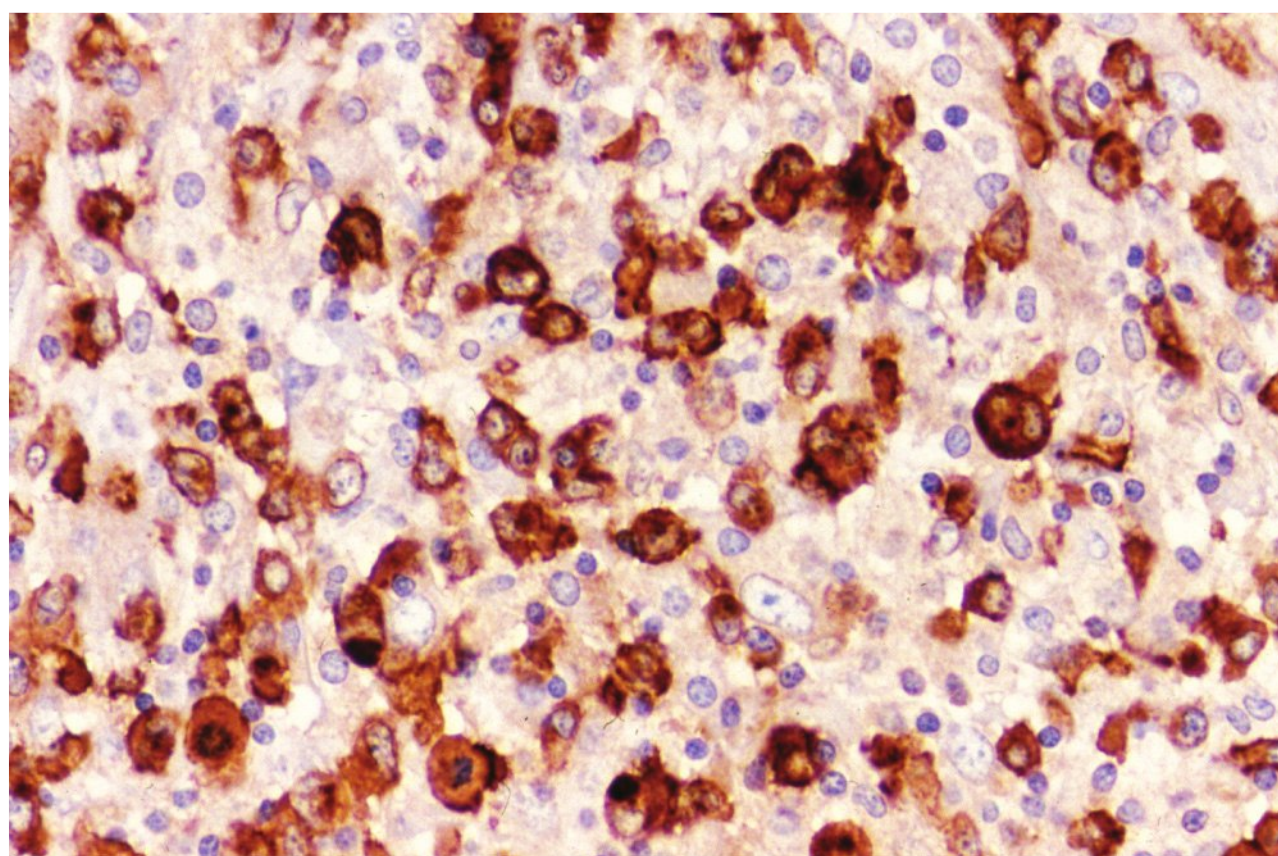


FIGURE 6.45.11 Case of small-cell pattern shows EMA staining in most of the tumor cells. Immunoperoxidase, 60× magnification.

An unexplainable phenomenon is the absence of T-cell receptor (TCR) protein on ALCL cells despite the presence of TCR gene rearrangement (27). The defective TCR expression in ALCL is probably analogous to the defective immunoglobulin expression in Hodgkin lymphoma (27).

A flow cytometric study of 19 cases of ALCL showed the expression of CD2 in 71% of cases, CD3 in 32%, CD4 in 63%, CD5 in 26%, CD7 in 32%, and CD8 in 21% (28). No matter whether CD4 or CD8 is predominant in a case, cytotoxic proteins, namely, T-cell-restricted intracellular antigen-1, granzyme B, and perforin, are frequently expressed, particularly in ALK-positive systemic ALCL (8,9,20). As CD56 is also expressed in a subset of ALCL cases and 10% of ALCL cases have no TCR gene rearrangement, there is a possibility that a minority of ALCL cases is derived from natural killer (NK) cells (13). Those CD56+ cases are associated with a worse prognosis (29). In addition to CD30, there are a few more activation antigens identified in ALCL, which include CD25 (interleukin-2 receptor), CD71 (transferrin receptor), and human leukocyte antigen-DR (2). The positive rate of CD25 in childhood ALCL is especially high (75%), but one study failed to demonstrate the relationship between CD25 and HTLV-1 infection in those cases (30).

CC-chemokine receptor 4 (CCR4) is expressed in primary cutaneous ALCL but not in systemic ALCL (19). In contrast, primary cutaneous ALCL does not express ALK, EMA, or clusterin in most cases. The use of CD15, BNH.9 (blood group antigen H and Y), EMA, ALK, clusterin, and cytotoxic proteins may help to distinguish ALCL from Hodgkin lymphoma, although overlapped results can be demonstrated in these two entities (2,8,9,20). Recently, the B-cell-specific activation protein (BSAP or PAX5) has been found to be expressed only by Reed-Sternberg cells but not by cells of T-cell or null cell-type ALCL (30). The distinctions between systemic and primary cutaneous ALCL and Hodgkin lymphoma are listed in Table 6.45.2 (8,9,20).

TABLE 6.45.2
Distinction between Systemic ALCL, Primary Cutaneous ALCL, and Hodgkin Lymphoma

Features	Systemic ALCL	Primary cutaneous ALCL	Hodgkin lymphoma
Gene rearrangement	TCR	TCR	Ig
Cytotoxic proteins	+	±	—
ALK	+	—	—
CD15	—	—	+
Clusterin	+	+	—
EMA	+	—	—
BSAP (PAX5)	—	—	+
BNH.9	+	—	—
EBV	—	—	±
CCR4	—	+	—

ALCL, anaplastic large-cell lymphoma; ALK, anaplastic lymphoma kinase; BNH.9, blood group antigen H and Y; BSAP, B-cell-specific activation protein; CCR4, CC-chemokine receptor 4; EBV, Epstein-Barr virus; EMA, epithelial membrane antigen; Ig, immunoglobulin gene; TCR, T-cell receptor gene.

Comparison of Flow Cytometry and Immunohistochemistry

The diagnosis of ALCL depends mainly on immunohistochemistry because most markers are available for this technique. Flow cytometry can demonstrate CD30 only when large numbers of tumor cells express this antigen. In addition, flow cytometry can show the selective loss of some T-cell markers. ALK protein can also be demonstrated by flow cytometry, as shown in a recent study (28).

Molecular Genetics

TCR gene analysis has shown that 90% of cases of ALCL have TCR b- and g-chain gene rearrangement, including those cases with a null cell immunophenotype (8,9). Some of the TCR negative cases may be of NK-cell origin, as suggested by the positive reaction to CD56 and the presence of cytotoxic proteins.

The recently discovered clusterin gene is specific to ALCL and is seen only in a minority of B-cell lymphomas (32). This gene is not found in Hodgkin lymphoma or other T-cell lymphomas.

In the late 1980s, a recurrent, reciprocal, balanced translocation, t(2;5)(p23;q35), was found in most cases of ALCL. This translocation leads to the fusion of the ALK gene at 2q23 and NPM at 5q35 (8,9). As a result, a chimeric NPM-ALK protein is produced. ALK is a novel tyrosine kinase, whereas NPM carries the newly synthesized protein from the cytoplasm to the nucleolus (9).

Although NPM-ALK fusion protein is demonstrated in 72.5% of cases of ALCL, other proteins are detected fusing with ALK as a result of other cytogenetic abnormalities (8,9,33,34). For instance, t(1;2)(q21;p32) produces nonmuscle tropomyosin (TPM3)-ALK fusion protein. Translocation

t(2;3)(p23;q21) leads to the product of tropomyosin receptor kinase-fusion gene (TRK)-ALK fusion protein. Inversion (2)(p23;q35) encodes 5-amino-imidazole-4-carboxamide-1-beta-D-ribofuranosyl transferase/inosine monophosphate cyclohydrolase (ATIC)-ALK fusion protein. Translocation t(2;17)(p23;q11) leads to the production of clathrin heavy polypeptide-like gene (CLTCL)-ALK fusion protein (7). Finally, t(2;X)(p23;q11-12) produces moesin (MSN)-ALK fusion protein (35). Additional partner genes, such as TPM-4 in t(2;19)(p23;q13.1), MYH9 in t(2;22)(p23;q11.12), AL017 in t(2;17)(p23;q25), have been discovered recently (5,6). These various fusion proteins other than NPM contain no nuclear localization signals, so they are absent in the nucleus and have only cytoplasmic distribution. The frequency of these cytogenetic variants and their products and distribution are summarized in Table 6.45.3 (12,13). A recent study of 21 pediatric cases of ALCL showed that 7 cases had t(2;5), 6 cases had variants, and 7 cases had uncharacterized rearrangements (36). The authors suggested that the frequency of variants in ALCL is probably higher than what has been conceived. The constant presence of the ALK gene in ALCL cases indicates its importance in the pathogenesis of ALCL. However, ALK gene does not work alone for tumorigenesis in ALCL; it involves a complex network of protein kinases, protein phosphatases, transcription factors, apoptosis and cell-cycle regulators, adaptor proteins, and other molecules to induce tumor formation (37).

The NPM-ALK translocation can be demonstrated by various techniques, including Southern blotting, reverse transcriptase-polymerase chain reaction (PCR), RNA in situ hybridization, and genomic DNA-PCR (38-40). These techniques are either time consuming, difficult to apply

TABLE 6.45.3

Characteristics of Various ALK Fusion Proteins

Frequency	Genetic abnormality	Fusion proteins	Staining patterns
72.5%	t(2;5)(p23;q35)	NPM-ALK	Cytoplasmic and nuclear
17.5%	t(1;2)(q25;p23)	TPM3-ALK	Cytoplasmic and membrane
2.5%	t(2;3)(p23;q21)	TFG-ALK	Cytoplasmic
2.5%	inv(2)(p23;q35)	ATIC-ALK	Cytoplasmic
2.5%	t(2;17)(p23;q11)	CLTCL-ALK	Granular cytoplasmic
<1.0%	t(2;X)(p23;q11-12)	MSN-ALK	Membrane
<1.0%	t(2;19)(p23;q13.1)	TPM4-ALK	Cytoplasmic
<1.0%	t(2;22)(p23;q11.2)	MYH9-ALK	Cytoplasmic
<1.0%	t(2;17)(p23;q25)	AL017-ALK	Cytoplasmic

ALK, anaplastic lymphoma kinase; NPM, nucleophosmin; TPM, tropomyosin; TFG, tropomyosin receptor kinase-fusion gene; ATIC, 5-aminoimidazole-4-carboxamide-1-beta-D-ribonucleotide transformylase/inosine monophosphate cyclohydrolase; CLTCL, clathrin heavy polypeptide-like gene; MSN, moesin.

to paraffin sections, or prone to artifact. Therefore, immunohistochemical staining with monoclonal or polyclonal antibodies against the fusion protein, NPM-ALK, appears to be the most desirable technique for a prompt diagnosis (9). However, molecular genetic techniques are important to identify the variants and additional cytogenetic aberrations.

A gene expression profiling (GEP) study showed that ALCL, ALK⁺ and ALCL, ALK⁻ tumors have distinct molecular signatures; overexpression of BCL-6, PTPN12, serpinA1, and C/EBP is found in ALCL, ALK⁺ and not in ALCL, ALK⁻ cases (41). Another GEP study found unexpected similarity in signatures between systemic and cutaneous ALCL, and between ALCL, ALK⁻, and classical Hodgkin lymphoma cases (42). GEP studies, however, have demonstrated clearly distinguished patterns in ALCL tumors that separate them from other peripheral T-cell neoplasms (43).

The current case is typical for a small-cell variant of ALCL in morphology showing predominantly small tumor cells with a minority of large tumor cells infiltrating the perivascular areas. Immunohistochemical studies are diagnostic by demonstrating positive staining of CD30, ALK, and EMA. The demonstration of t(2;5) translocation further confirms the diagnosis. Flow cytometry demonstrated CD30 and the selective loss of CD7. The low percentages of B-cell markers are helpful in substantiating the diagnosis of a T-cell lymphoma. The salient features of laboratory diagnosis of ALCL are summarized in Table 6.45.4.

Clinical Manifestations

Clinically, ALCL can be subdivided into primary and secondary forms (7,8). The primary form is further divided into systemic and cutaneous subforms. The primary form can be rarely induced by human immunodeficiency virus

infection, but those cases are usually of B-cell lineage. The clinical manifestation of ALCL varies greatly from case to case in early studies. The variation may be due to the differences of diagnostic criteria, the inclusion of cutaneous and systemic forms, the variation of patient age, and the immunophenotype. However, current studies have found that the most important factor that influences the clinical outcome is the presence or the absence of ALK protein (15,22,44-47). There are no clinical differences among various fusion proteins, whether it is NPM-ALK or its variants (2,7). Therefore, it appears that the variation of

TABLE 6.45.4

Salient Features of Laboratory Diagnosis of Systemic ALCL*

1. T-cell or null cell phenotype with predominance of T-helper cell subtype
2. Positive immunohistochemical staining for CD30, clusterin, EMA, cytotoxic proteins, and in most cases, ALK1
3. Negative for B-cell markers, BSAP and CD15
4. Cytogenetic or molecular demonstration of t(2;5) or NPM-ALK genes or their variants
5. TCR-b- or g-chain gene rearrangement is seen in most cases.

*See Table 6.45.2 for differences between systemic ALCL and primary cutaneous ALCL.

ALK, anaplastic lymphoma kinase; BSAP, B-cell-specific activation protein; EMA, epithelial membrane antigen; NPM, nucleophosmin.

cytogenetics does not affect the clinical course of ALCL. Accordingly, primary systemic ALCL is subdivided into ALK-positive and ALK-negative subforms.

Anaplastic Lymphoma Kinase–Positive Primary Systemic Anaplastic Large-Cell Lymphoma

ALK-positive systemic ALCL is usually seen in children and younger populations in their second and third decades (7,8). Its frequency is about 13% of childhood non-Hodgkin lymphoma and 2% of adult cases (2). The male/female ratio is 6.5:1. At diagnosis, the lymphoma is usually at stage III or IV. The noncontiguous distribution of lymphadenopathy, low frequency of splenomegaly, and high frequency of extranodal involvement help to distinguish ALCL from Hodgkin lymphoma (2).

Systemic B symptoms (fever, night sweats, and/or weight loss) are present in about 75% of patients. Extranodal involvement is commonly present in the skin, bone, soft tissues, lung, and liver (2,7,8). Bone marrow involvement is detected in about 11% of cases by examining hematoxylin and eosin–stained sections. With immunohistochemical stain, the positive rate increases to 30%. Peripheral blood involvement is uncommon in ALCL. Cases with leukemic presentation usually show the small-cell pattern and the t(2;5)(p23;q35) translocation and have a poor response to therapy or early relapse (48,49).

The 5-year survival rate is markedly different between the ALK-positive and the ALK-negative groups. In three studies, the differences were 79.8% versus 32.9% (45), 71% ± 8% versus 15% ± 11% (46), and 79% versus 46% (47), respectively.

Anaplastic Lymphoma Kinase–Negative Primary Systemic Anaplastic Large-Cell Lymphoma

ALK-negative systemic ALCL occurs in older individuals aged 46 to 61 years (7,8). The male/female ratio is 0.9:1. One study showed that this clinical form has a lower incidence of stage II to IV disease and extranodal involvement (46), but these findings were not confirmed by another study (47). However, both studies revealed a poor prognosis in this clinical form. Nevertheless, more recent studies revealed that this subtype has variable clinical behavior and that new parameters are needed to stratify this group of patients. The prognostic factors that are suggested include the International Prognostic Index (composed of age, disease stage, number of extranodal sites, lactate dehydrogenase level, and performance status) and the number of tumor-infiltrating activated cytotoxic T lymphocytes (19).

Primary Cutaneous Anaplastic Large-Cell Lymphoma

This form is invariably ALK negative. It is seen in older patients with a median age of about 60 years (7,8). The most common clinical presentation is a solitary, asymptomatic cutaneous or subcutaneous reddish-violet mass with or without superficial ulceration. The lesion is often located in the extremities and trunk (2). In contrast to the general rule, the ALK-negative primary cutaneous ALCL

has a better prognosis than does the ALCL, ALK+ with skin involvement. Approximately 25% of patients show partial or complete spontaneous regression. Patients have long-term survival after local excision with or without radiation therapy.

The histologic features of primary cutaneous ALCL overlap with those of lymphomatoid papulosis, and their distinction frequently depends on clinical follow-up. Almost 100% of cases of lymphomatoid papulosis have spontaneous regression (2). Therefore, no treatment is required for this entity, even with clinical relapse.

Secondary Anaplastic Large-Cell Lymphoma

Secondary ALCL represents a transformation from other lymphomas (mycosis fungoides, peripheral T-cell lymphomas), Hodgkin lymphoma, or lymphomatoid papulosis (7,8). It is usually seen in older individuals with the absence of ALK protein expression. The clinical prognosis is generally poor.

REFERENCES

1. Stein H, Mason DY, Gerdes J, et al. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and nonplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. *Blood*. 1985;66:848–858.
2. Kadin ME, Carpenter C. Systemic and primary cutaneous anaplastic large cell lymphomas. *Semin Hematol*. 2003;40:244–256.
3. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma study Group. *Blood*. 1994;84:1361–1392.
4. Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization classification of hematological malignancies report of the Clinical Advisory Committee meeting. Airlie House, Virginia, November 1997. *Mod Pathol*. 2000;13:193–207.
5. Delsol G, Falini B, Muller-Hermelink HK, et al. Anaplastic large cell lymphoma (ALCL), ALK-positive. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:312–316.
6. Mason DY, Harris NL, Delsol G, et al. Anaplastic large cell lymphoma, ALK-negative. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:317–319.
7. Delsol G, Ralfkiaer E, Stein H, et al. Anaplastic large cell lymphoma. In: Jaffe ES, Harris NL, Stain H, Vardiman JW, eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:230–235.
8. Anagnostopoulos I, Dallenback F, Stein H. Diffuse large cell lymphomas. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:855–913.
9. Stein H, Foss HD, Durkop H, et al. CD30+ anaplastic large cell lymphoma: a review of its histopathologic genetic and clinical features. *Blood*. 2000;96:3681–3695.
10. Kinney MC, Kadin ME. The pathologic and clinical spectrum of anaplastic large cell lymphoma and correlation with ALK gene dysregulation. *Am J Clin Pathol*. 1999;111(suppl 1):S56–S67.

11. Benharroch D, Meguerian-Bedoyan Z, Lamant L, et al. ALK-positive lymphoma. A single disease with a broad spectrum of morphology. *Blood*. 1998;91:2076–2084.
12. Kadin ME. Anaplastic large cell lymphoma and its morphological variants. *Cancer Surv*. 1997;30:77–86.
13. Chan JKC, Buchanan R, Fletcher CDM. Sarcomatoid variant of anaplastic large cell lymphoma. *Am J Surg Pathol*. 1990;14:383–390.
14. Mann KP, Hall B, Kamino H, et al. Neutrophil-rich, Ki-1 positive anaplastic large cell malignant lymphoma. *Am J Surg Pathol*. 1995;19:407–416.
15. McCluggage WG, Walsh MY, Bharucha H. Anaplastic large cell malignant lymphoma with extensive eosinophilic or neutrophilic infiltration. *Histopathology*. 1998;32:110–115.
16. Falini B, Liso A, Pasqualucci L, et al. CD30+ anaplastic large cell lymphoma, null type, with signet ring appearance. *Histopathology*. 1997;30:90–92.
17. Kinney MC, Collins RD, Greer JP, et al. A small cell predominant variant of primary Ki-1 (CD30)+ T-cell lymphoma. *Am J Clin Pathol*. 1993;17:859–868.
18. Pileri S, Falini B, Delsol G, et al. Lymphohistiocytic T-cell lymphoma (anaplastic large cell lymphoma CD30+/Ki-1+ with a high content of reactive histiocytes). *Histopathology*. 1990;16:683–391.
19. Cheuk W, Hill RW, Bacchi C, et al. Hypocellular anaplastic large cell lymphoma mimicking inflammatory lesions of lymph nodes. *Am J Surg Pathol*. 2000;24:1537–1543.
20. ten Berge RL, Oudejans JJ, Ossenkoppele GJ, et al. ALK-negative systemic anaplastic large cell lymphoma: differential diagnostic and prognostic aspects—a review. *J Pathol*. 2003;200:4–15.
21. Simonart T, Kentos A, Renotirte C, et al. Cutaneous involvement by neutrophil-rich, CD30-positive anaplastic large cell lymphoma mimicking deep pustules. *Am J Surg Pathol*. 1999;23:244–246.
22. Haralambieva E, Pulford K, Lamant L, et al. Anaplastic large cell lymphomas of B-cell phenotype are anaplastic lymphoma kinase (ALK) negative and belong to the spectrum of diffuse large B-cell lymphomas. *Br J Haematol*. 2000;109:584–591.
23. Pulford K, Lamant L, Morris SW, et al. Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood*. 1997;89:1394–1404.
24. Nascimento AF, Pinkus JL, Pinkus GS. Clusterin, a marker for anaplastic large cell lymphoma: immunohistochemical profile in hematopoietic and nonhematopoietic malignant neoplasms. *Am J Clin Pathol*. 2004;121:709–717.
25. Olsen SH, Ma L, Schnitzer B, et al. Clusterin expression in cutaneous CD30-positive lymphoproliferative disorders and their histologic stimulants. *J Cutan Pathol*. 2009;36:302–307.
26. Falini B, Pileri S, Stein H, et al. Variable expression of leukocyte-common (CD45) antigen in CD30 (Ki-1)-positive anaplastic large-cell lymphoma: implications for the differential diagnosis between lymphoid and non-lymphoid malignancies. *Hum Pathol*. 1990;21:624–629.
27. Bonzheim I, Geissinger D, Roth S, et al. Anaplastic large cell lymphomas lack the expression of T-cell receptor molecules or molecules of proximal T-cell receptor signaling. *Blood*. 2004;104:3358–3360.
28. Juco J, Holden JT, Mann KP, et al. Immunophenotypic analysis of anaplastic large cell lymphoma by flow cytometry. *Am J Clin Pathol*. 2003;119:205–212.
29. Suzuki R, Kagami Y, Takeuchi K, et al. Prognostic significance of CD56 expression for ALK-positive and -negative anaplastic large cell lymphoma of T/null cell phenotype. *Blood*. 2000;96:2993–3000.
30. Gualco G, Chioato L, Weiss LM, et al. Analysis of human T-cell lymphotropic virus in CD25+ anaplastic large cell lymphoma in children. *Am J Clin Pathol*. 2009;132:28–33.
31. Foss HD, Reusch R, Demel G, et al. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. *Blood*. 1999;94:3108–3113.
32. Wellmann A, Thieblemont C, Pittaluga S, et al. Detection of differentially expressed genes in lymphomas using cDNA arrays: identification of clusterin as a new diagnostic marker for anaplastic large cell lymphomas. *Blood*. 2000;96:398–404.
33. Falini B, Pulford K, Pucciarini A, et al. Lymphomas expressing ALK fusion protein(s) other than NPM-ALK. *Blood*. 1999;94:3509–3515.
34. Dresler HG, Gignac SM, von Wasielewski R, et al. Pathobiology of NPM-ALK and variant fusion genes in anaplastic large cell lymphoma and other lymphomas. *Leukemia*. 2000;14:1533–1559.
35. Tort F, Pinyol M, Pulford K, et al. Molecular characterization of a new ALK translocation involving moesin (MSN-ALK) in anaplastic large cell lymphoma. *Lab Invest*. 2001;81:419–426.
36. Liang X, Meech SJ, Odom LF, et al. Assessment of t(2;5) (p23;q35) translocation and variants in pediatric ALK+ anaplastic large cell lymphoma. *Am J Clin Pathol*. 2004;121:496–506.
37. Amin HM, Lai R. Pathobiology of ALK+ anaplastic large-cell lymphoma. *Blood*. 2007;110:2259–2267.
38. Cataldo KA, Jalal SM, Law ME, et al. Detection of t(2;5) in anaplastic large cell lymphoma. Comparison of immunohistochemical studies, FISH, and RT-PCR in paraffin-embedded tissue. *Am J Surg Pathol*. 1999;23:1386–1392.
39. Johnson PW, Leek J, Swinbank K, et al. The use of fluorescent in situ hybridization for detection of the t(2;5) (p23;q35) translocation in anaplastic large cell lymphoma. *Ann Oncol*. 1997;8(suppl 2):65–69.
40. Tai YC, Kim LH, Peh SC. Common ALK gene rearrangement in Asian CD30+ anaplastic large cell lymphoma: an immunohistochemical and fluorescence in situ hybridization (FISH) study on paraffin-embedded tissue. *Pathology*. 2003;35:436–443.
41. Lamant L, de Reynies A, Duplantier MM, et al. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+ subtypes. *Blood*. 2007;109:2156–2164.
42. Eckerle S, Brune V, Doring C, et al. Gene expression profiling of isolated tumour cells from anaplastic large cell lymphomas: insights into its cellular origin, pathogenesis and relation to Hodgkin lymphoma. *Leukemia*. 2009;23:2129–2138.
43. Piva R, Agnelli L, Pellegrino E, et al. Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. *J Clin Oncol*. 2010;28:1583–1590.
44. Tilly H, Gaulard P, Lepage E, et al. Primary anaplastic large-cell lymphoma in adults: clinical presentation, immunophenotype, and outcome. *Blood*. 1997;90:3727–3734.
45. Shiota M, Nakamura S, Ichinohasama R, et al. Anaplastic large cell lymphomas expressing the chimeric protein p80/NPM/ALK: a distinct clinicopathologic entity. *Blood*. 1995;86:1954–1960.

46. Falini B, Pileri S, Zinzani PL, et al. ALK+ lymphoma: clinicopathological findings and outcome. *Blood*. 1999;93:2697–2706.
47. Gascoyne R, Aour P, Wu D, et al. Prognostic significance of anaplastic lymphoma kinase (ALK) protein expression in adults with anaplastic large cell lymphoma. *Blood*. 1999;93:3913–3921.
48. Onciu M, Behm FG, Raimondi SC, et al. ALK-positive anaplastic large cell lymphoma with leukemic peripheral

- blood involvement is a clinicopathologic entity with an unfavorable prognosis. Report of three cases and review of the literature. *Am J Clin Pathol*. 2003;120:617–625.
49. Nguyen JT, Condrón MR, Nguyen ND, et al. Anaplastic large cell lymphoma in leukemia phase: Extraordinarily high white blood cell count. *Pathol Int*. 2009;59:345–353.

CASE 46

Primary Cutaneous Anaplastic Large-Cell Lymphoma

CASE HISTORY

An 86-year-old man presented with an ulcerated nodular mass on the right lower leg for a few months. The patient had a long history of psoriasis/dermatitis for 40 years and had been treated intermittently with psoralen plus ultraviolet A. However, the skin lesion grew rapidly on the right lower leg for the past few months prior to admission and became an ulcerative nodular mass. He also reported 20-lb weight loss over the past few months. He had increasing fatigue and frequently woke up with night sweats, but he did not have nausea, vomiting, and fever. The patient had a history of colon carcinoma about 20 years ago.

Physical examination on admission revealed a 4 × 8 cm ulcerative fungating mass with peripheral erythema on the right lower leg. No hepatosplenomegaly or lymphadenopathy was detected. Laboratory examination showed a total leukocyte count of 7,000/mL with 73.2% neutrophils, 19.3% lymphocytes, 5.5% monocytes, 1.5% eosinophils, and 0.5% basophils. His hemoglobin was 13.7 g/dL, hematocrit 45.5%, and platelets 285,000/mL. Blood chemistry panel revealed normal levels of lactate dehydrogenase and creatinine.

Skin biopsy was performed that showed anaplastic large cells infiltrating the dermis and subcutis (Figs. 6.46.1–6.46.3). Staging work-up revealed no systemic disease. The patient was treated with local x-ray and one cycle of CVP. Then he developed diarrhea, but culture and toxin test for *Clostridium difficile* were negative. During the hospital course, his diarrhea was finally under control with metronidazole. The patient refused to have further chemotherapy and was transferred to a hospice inpatient facility.

IMMUNOHISTOCHEMISTRY

Immunohistochemical stains with appropriate controls showed that the large tumor cells were positive for CD3 (Fig. 6.46.4) and CD30 (Fig. 6.46.5), but were negative for CD20 (Fig. 6.46.6), ALK1 (Fig. 6.46.7), CD21 (Fig. 6.46.8), and CD15.

DISCUSSION

Primary cutaneous anaplastic large-cell lymphoma (PCALCL) is defined by the World Health Organization-European Organization for Research and Treatment of Cancer (WHO-EORTC) classification as a neoplasm composed of large cells with an anaplastic, pleomorphic, or immunoblastic cytomorphology and expression of the CD30 antigen by >75% of the tumor cells (1). The WHO-EORTC includes lymphomatoid papulosis (LyP) and PCALCL in a single category, designated primary cutaneous CD30+ lymphoproliferative disorders, which account for approximately 30% of cutaneous T-cell lymphomas. The 2008 WHO classification adopts this nomenclature with the addition of a borderline group (2). The WHO classification also emphasizes the exclusion of mycosis fungoides (MF)–transformed cutaneous CD30+ lymphoma and systemic anaplastic large-cell lymphoma (SALCL) from the category of primary cutaneous CD30+ lymphoproliferative disorders.

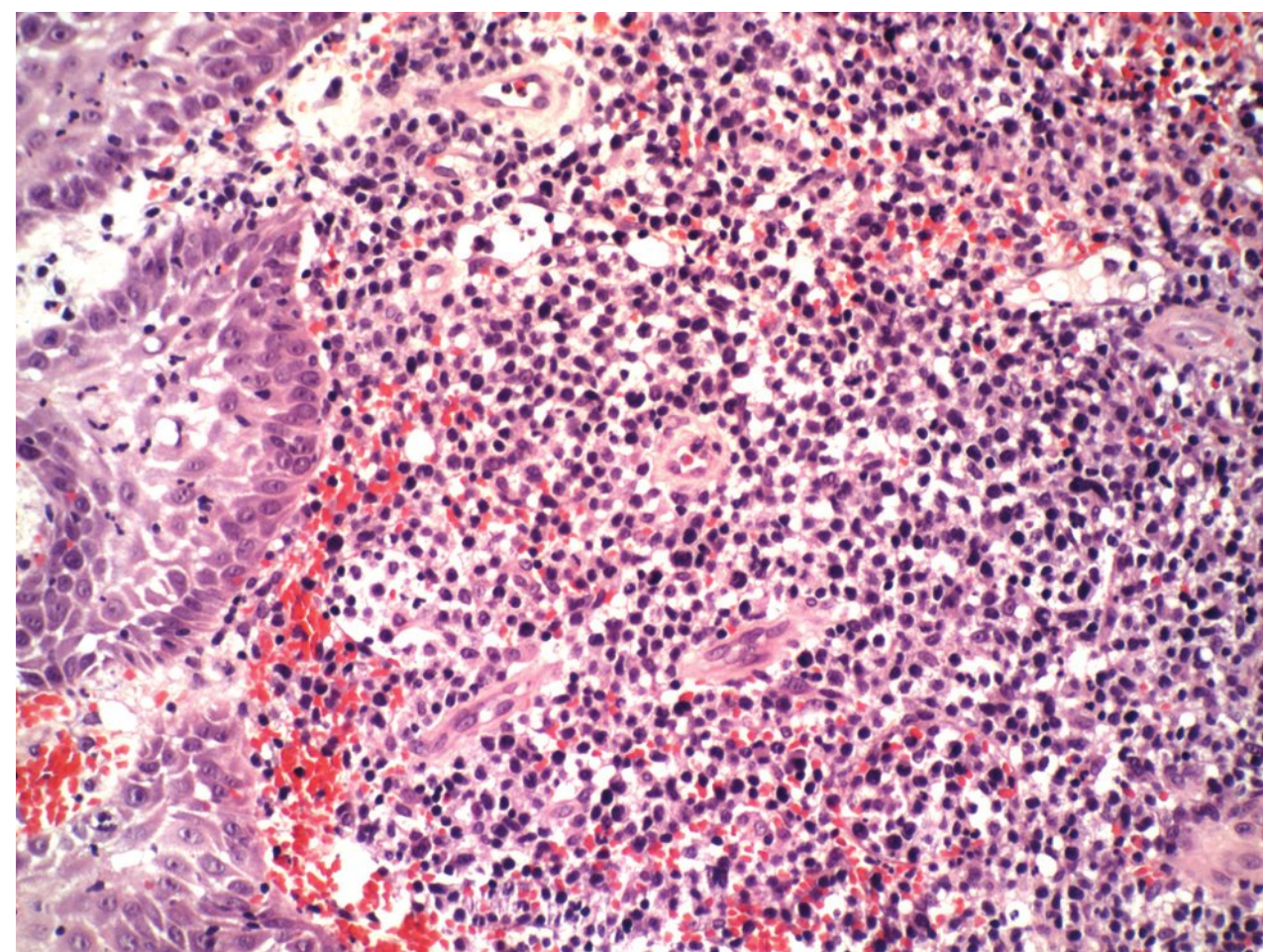


FIGURE 6.46.1 Skin biopsy shows extensive cellular infiltration in the dermis. H&E, ×20.

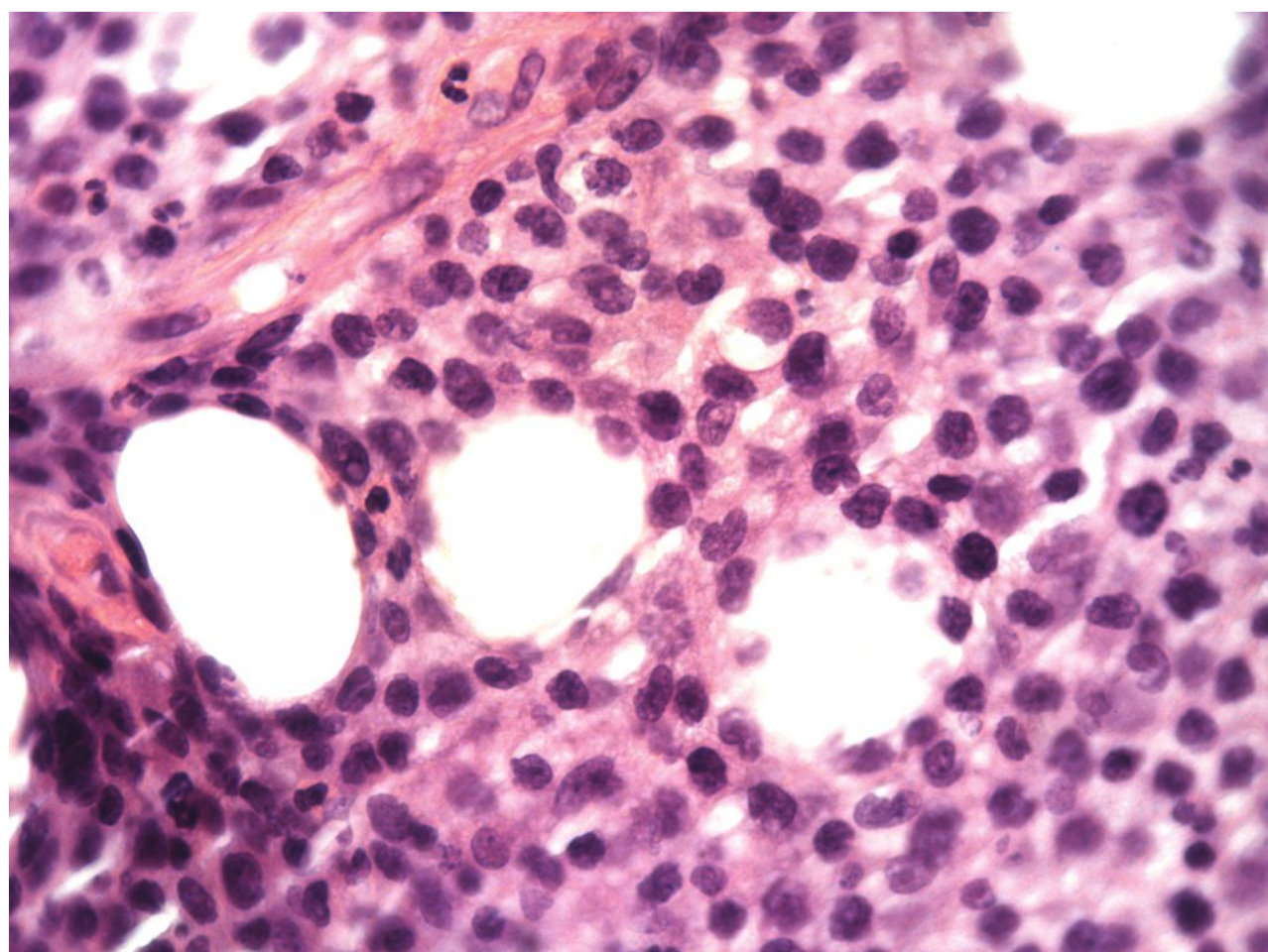


FIGURE 6.46.2 Skin biopsy reveals involvement of subcutis by anaplastic large cells. H&E, ×60.

Morphology

PCALCL usually involves the dermis and the subcutis with the epidermis spared (1–3). The infiltration is frequently perivascular and periappendiceal as well as involvement of collagen fibers. A cohesive infiltration with sheets or clusters of tumor cells is characteristic of anaplastic large-cell lymphoma. Occasionally, angioinvasion is demonstrated that helps to distinguish PCALCL from LyP (3). In the majority of cases, the tumor cells show an anaplastic cytomorphology, showing large tumor cells with abundant cytoplasm, immature chromatin pattern with or without prominent nucleoli. Similar to the hallmark cells in SALCL, kidney-shaped or horseshoe-like nucleus is frequently identified. Reed-Sternberg-like cells can also be seen. In 20% to 25% of cases, the tumor cells appear to be pleomorphic or immunoblastic (1,2). A small-cell variant with epidermotropism has also been reported in a few cases (4,5).

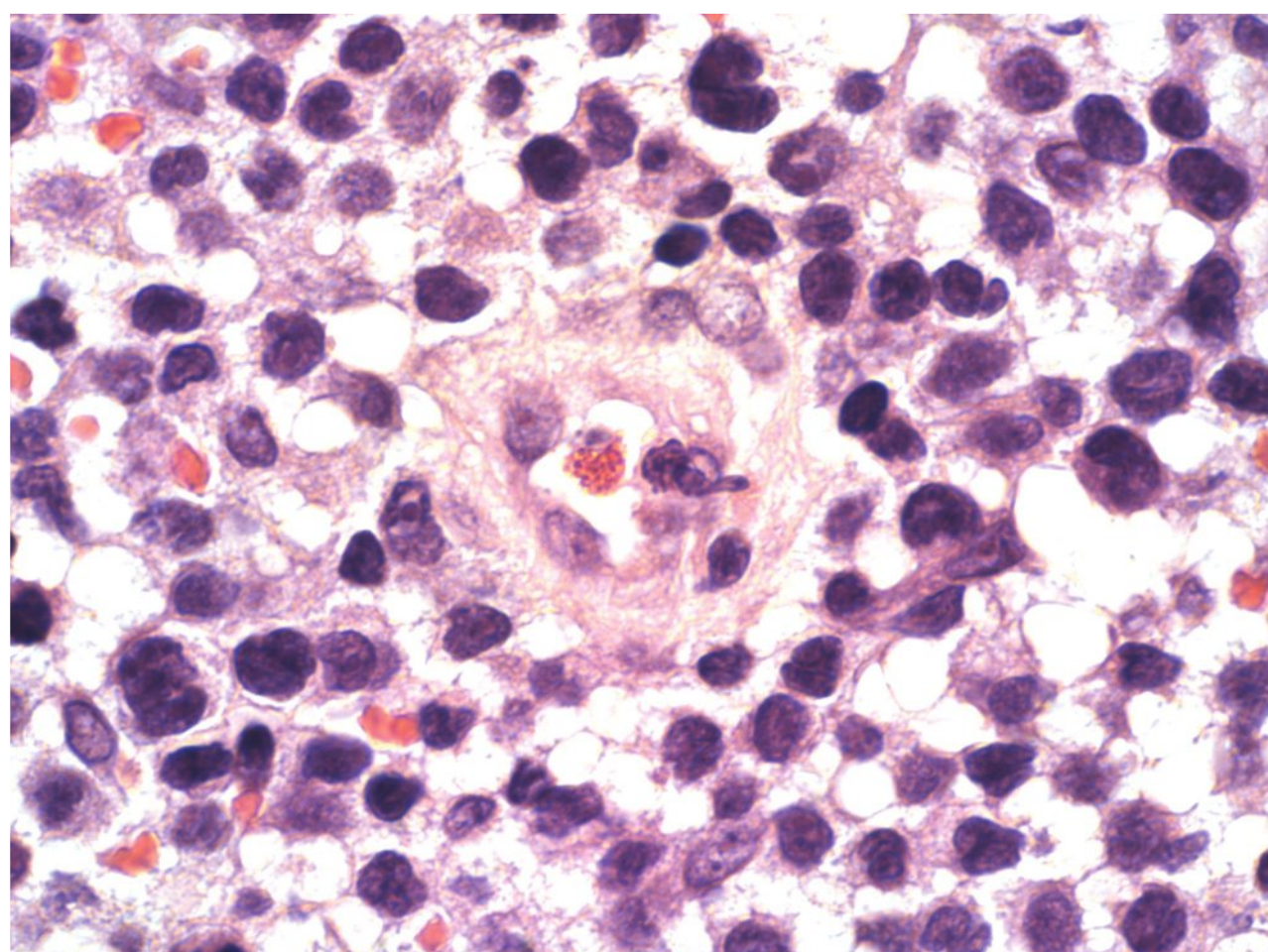


FIGURE 6.46.3 Higher magnification shows anaplastic large cells with a few kidney-shaped nuclei. H&E, ×100.

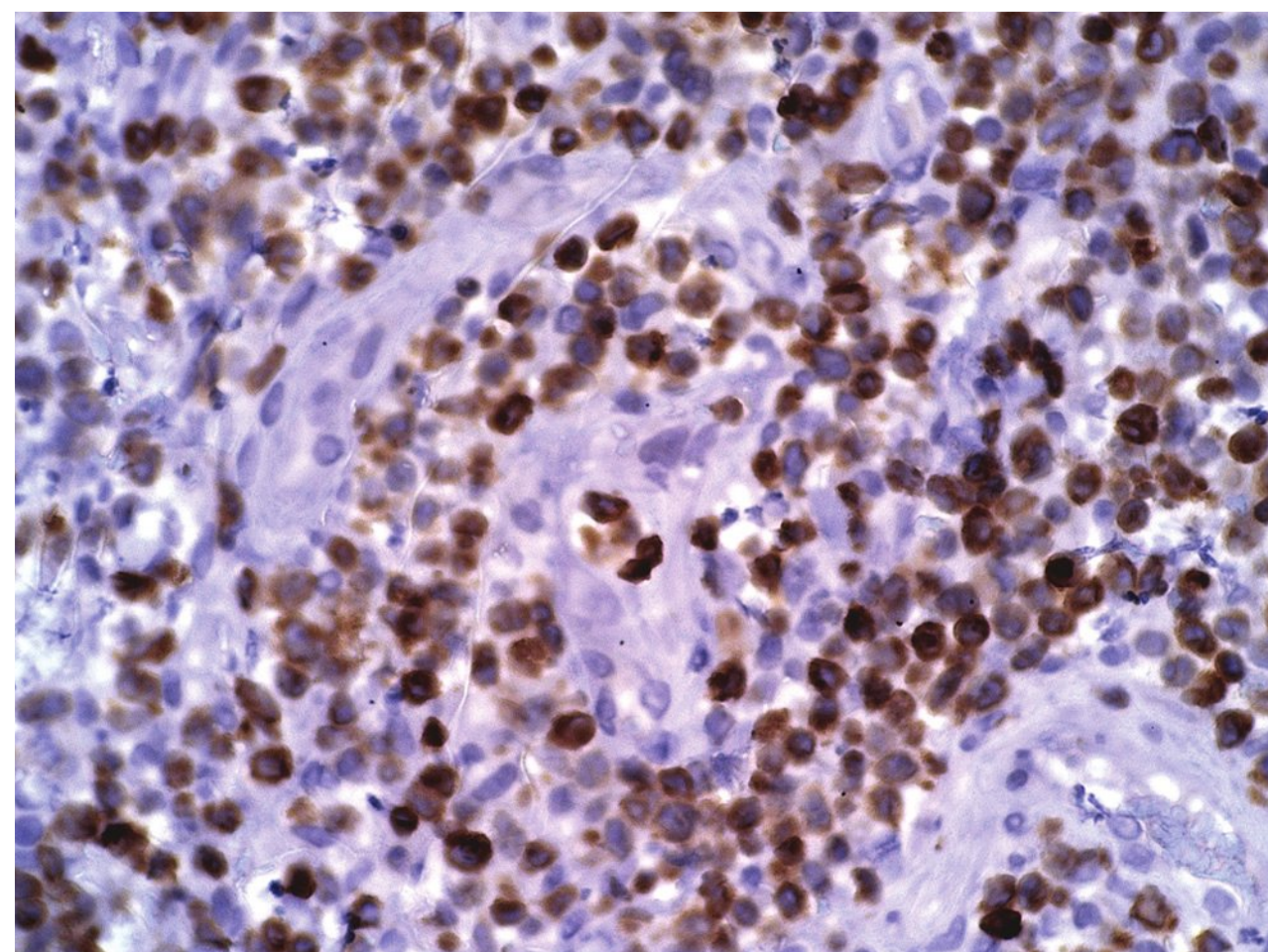


FIGURE 6.46.4 CD3 stain is positive for most of the tumor cells. Immunoperoxidase, ×40.

In most cases of PCALCL, inflammatory cells are rare, which is a distinguishing feature from LyP. However, when ulceration is present in the lesion, inflammatory cells, such as eosinophils, neutrophils, histiocytes, and reactive T cells, can be predominant and mask the large CD30+ tumor cells (1,2). In addition, there are rare cases of neutrophil-rich or neutrophil/eosinophil-rich PCALCL reported (6–9). These cases may have abscess formation and was termed by Burg et al. (10) as pyogenic cutaneous lymphoma and should be differentiated from infectious diseases. One report mentioned the presence of large numbers of Langerhans cells in a case of PCALCL, mimicking Langerhans cell histiocytosis (11). Cepeda et al. (12) demonstrated CD30-positive atypical lymphoid cells in many non-neoplastic cutaneous lesions, including spider bite, herpes simplex, hidradenitis, ruptured cyst, rhinophyma, and stasis ulcer. CD30 is a member of the tumor necrosis

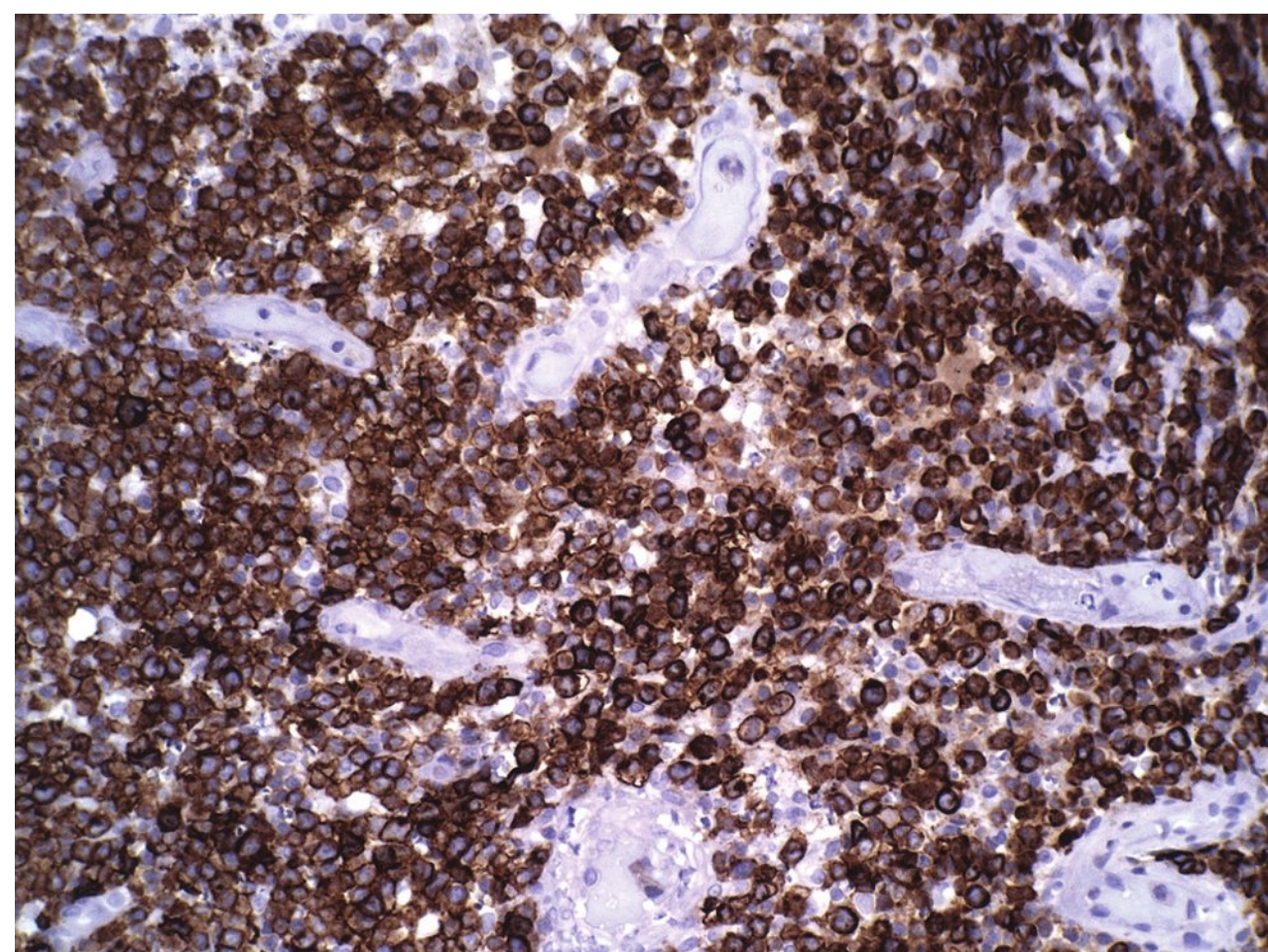


FIGURE 6.46.5 CD30 stain is positive for all tumor cells. Immunoperoxidase, ×20.

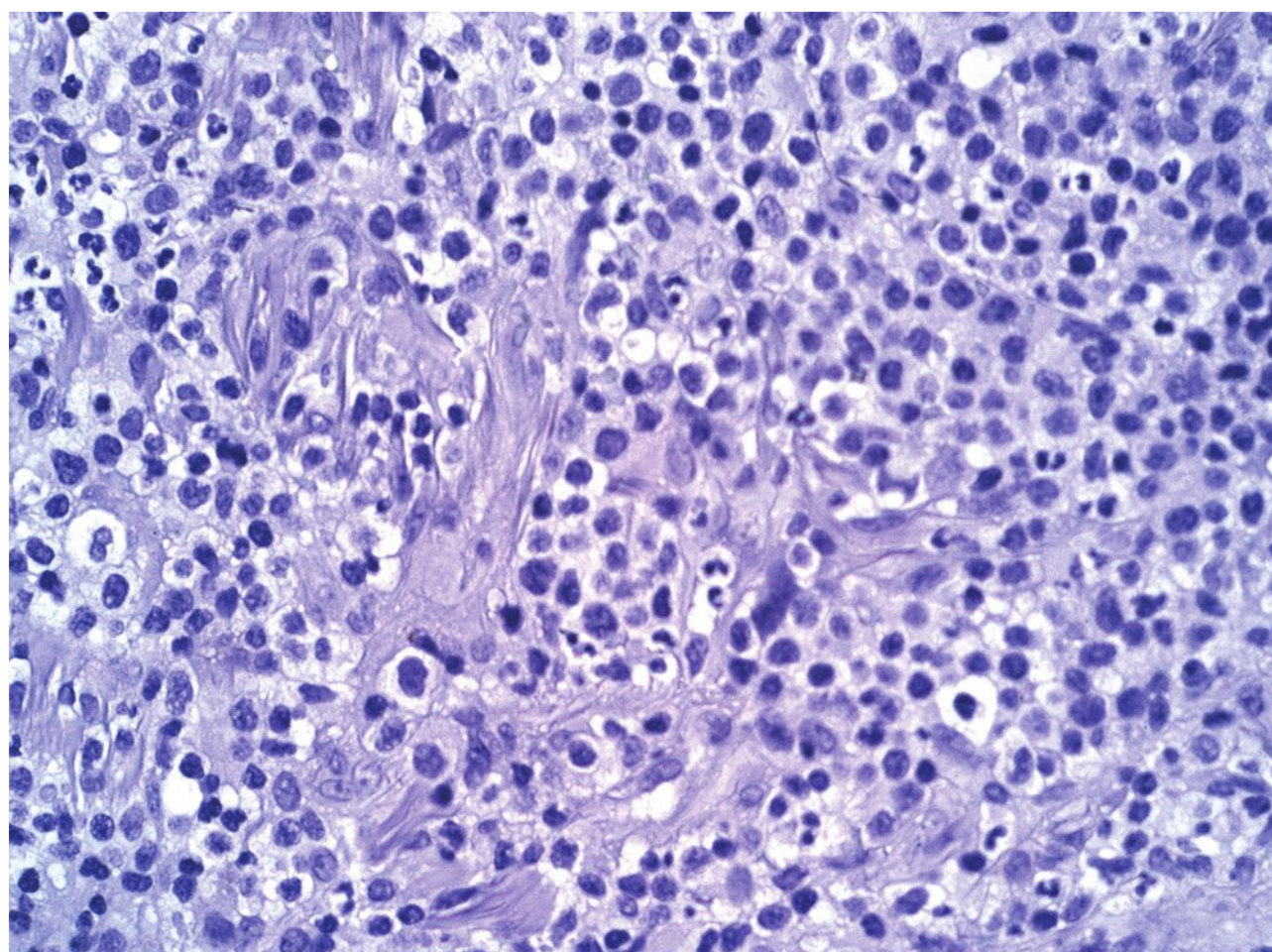


FIGURE 6.46.6 CD20 stain is negative for tumor cells. Immunoperoxidase, $\times 40$.

factor/nerve growth factor receptor superfamily, thus can be detected in activated non-neoplastic B and T cells albeit in a low percentage.

The major differential diagnosis is LyP, which is very similar to PCALCL in terms of morphology, immunophenotype, and molecular genetics. The clinical presentation is most helpful in the differential diagnosis. As mentioned before, a mild inflammatory reaction or a vascular invasion by tumor cells may help to separate PCALCL from LyP.

PCALCL is indistinguishable from SALCL by morphology, but the clinical course and prognosis are quite different in these two entities. Therefore, a thorough staging workup should be conducted to exclude systemic disease before a diagnosis of PCALCL is made. A comprehensive immunophenotyping and genotyping may help separate these two diseases in most cases. In cases that show Reed-Sternberg-like cells, cutaneous Hodgkin lymphoma should be excluded by immunophenotyping. If the patient has a

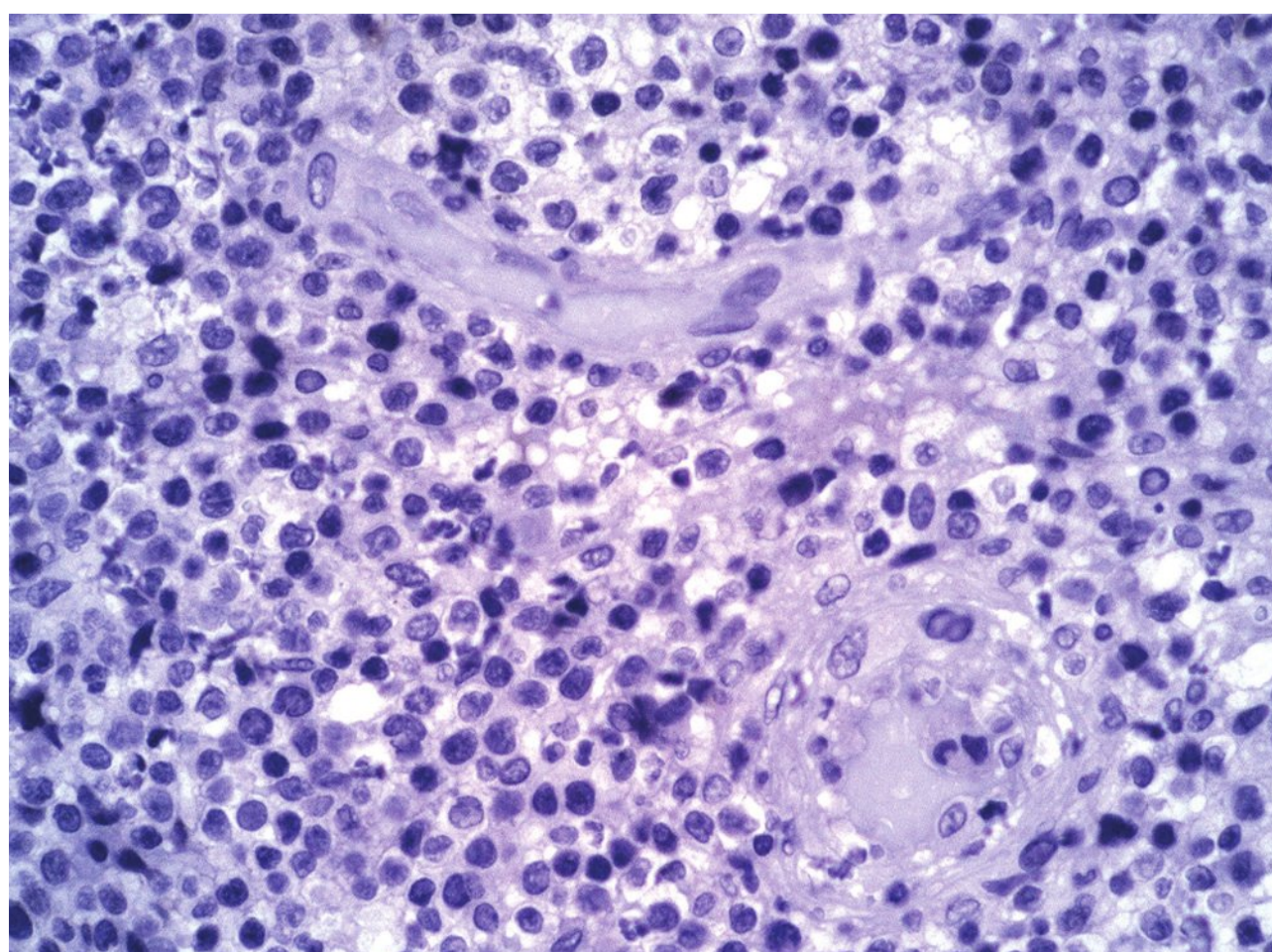


FIGURE 6.46.7 ALK stain is negative for tumor cells. Immunoperoxidase, $\times 40$.

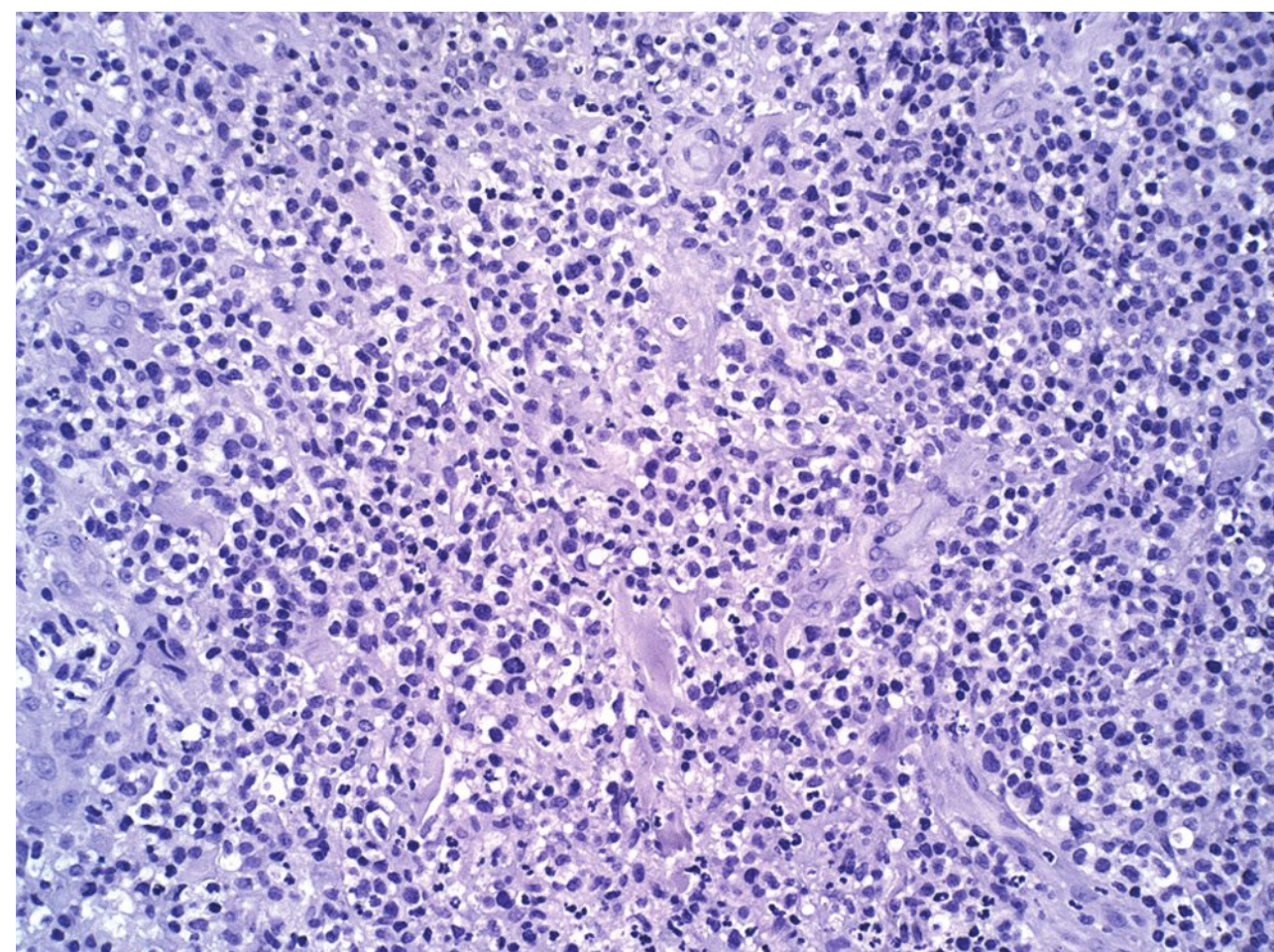


FIGURE 6.46.8 CD21 stain shows no follicular dendritic cell meshwork. Immunoperoxidase, $\times 10$.

previous history or current clinical features of MF, the case should be considered the tumor stage of MF rather than PCALCL, as those tumors may be CD30+.

Immunophenotype

By definition, PCALCL should express CD30 in more than 75% of tumor cells. Most cases are of T-helper cell phenotype (CD3+, CD4+, CD8-), but CD4-, CD8+ and CD4-, CD8- phenotypes have been reported in a minority of cases (9). Cytotoxic proteins (granzyme B, TIA-1, perforin) are frequently present even in cases of T-helper cell type. Selective loss of T-cell markers, such as CD2, CD3, or CD5, has been demonstrated in variable numbers of cases.

The most reliable markers to distinguish PCALCL from SALCL are the anaplastic large-cell kinase (ALK) and epithelial membrane antigen (EMA), which are positive in most cases of SALCL but negative in cases of PCALCL (1-3). However, an exceptional case of ALK/EMA-positive PCALCL has been reported (13). Clusterin was considered specific marker for SALCL in the early literature, but subsequent studies identified clusterin in up to 50% to 67% of PACLCL cases (14-16) and in all 4 pediatric cases in one study (17). Clusterin is also expressed in 50% of LyP, and 75% of MF with large-cell transformation; therefore, it is not helpful in the differential diagnosis (16). In the study by ten Berge et al. (18), CC-chemokine receptor 4 was only demonstrated in PCALCL but not in ALK+ SALCL. However, no subsequent study of this marker has been reported.

Cutaneous lymphocyte-associated antigen (CLA) had been considered a marker for distinction between PCALCL and SALCL, but it was demonstrated in both primary and secondary cutaneous ALCL, and not in nodal ALCL without skin involvement in one study (19). Multiple myeloma oncogene 1 (MUM1) was considered to be discriminative between LyP and PCALCL in one study (20), but was found positive for both entities in other two studies (21,22). Tumor necrosis factor receptor-associated factor 1 (TRAF1) also showed no value in the distinction between LyP and PCALCL (23). CD15 is important in exclusion of

TABLE 6.46.1

Comparison between PCALCL and SALCL

	PCALCL	SALCL
CD30	Positive	Positive
ALK	Negative	Positive/negative
EMA	Negative	Positive
Clusterin	50% case positive	>90 cases positive
CLA	Positive	Negative in lymph node
CD4	Positive	Positive*
CD8	Negative	Negative
Cytotoxic proteins	Positive	Positive
CCR4	Positive	Negative
EBV	Negative	Negative
TCR rearrangement	Positive	Positive
t(2;5) translocation	Negative	Positive

*In rare cases of null cell type, T-cell markers are negative.

ALK, anaplastic lymphoma kinase; CCR4, CC-chemokine receptor 4; CLA, cutaneous lymphocyte-associated antigen; EBV, Epstein-Barr virus; EMA, epithelial membrane antigen; PCALCL, primary cutaneous anaplastic large-cell lymphoma; SALCL, systemic anaplastic large-cell lymphoma; TCR, T-cell-receptor gene.

the diagnosis of Hodgkin lymphoma, but it was identified in 43% of PACLCL cases compared with 18% of LyP in one study (23). In the same study, BCL-2 was expressed in 21% of PCALCL and 36% of LyP cases (23).

CD45, another marker used to distinguish Hodgkin lymphoma from non-Hodgkin lymphoma, can be negative or weakly positive in PACLCL, so it is not reliable for differential diagnosis (17). CD99 was strongly positive for three of four cases of PACLCL in one study, but it can be positive for precursor hematopoietic neoplasms and other peripheral T-cell lymphomas (17). Coexpression of CD56 with CD30 has been shown in rare cases of PCALCL, but it does not confer an unfavorable prognosis (24). Epstein-Barr virus (EBV) was demonstrated by EBV encoded RNA in situ hybridization (EBER) or latent membrane protein-1 in 2 of 16 cases of PCALCL in a Korean study; both cases showed extracutaneous dissemination (25). The difference between primary and secondary cutaneous anaplastic large-cell lymphoma is listed in Table 6.46.1.

Comparison between Flow Cytometry and Immunohistochemistry

Flow cytometry is able to demonstrate a T-helper cell immunophenotype (CD3+, CD4+, CD8-) with the expression of CD30, but it does not help to distinguish primary from secondary cutaneous anaplastic large-cell lymphoma. Immunohistochemical stains may demonstrate the presence of CLA and the absence of ALK, EMA, and CD15, which help to distinguish PCALCL from SALCL and from Hodgkin lymphoma. In the rare cases of CD15+, PCALCL, PAX5/BSAP staining should be added for the distinction from Hodgkin lymphoma, which is positive for this protein.

Marker studies, however, play no role in the exclusion of LyP.

Molecular Genetics

Most cases of PACLCL have monoclonal T-cell receptor (TCR) gene rearrangement, which does not differ from the SALCL. One study found that clonal TCR gene rearrangement was positive in 11/17 cases PCALCL, none of 10 LyP type A and B, and 4/8 cases of LyP type C by polymerase chain reaction on archival tissue specimens (26). The absence of t(2;5)(p23;q35) by karyotyping or of ALK-NPM (nucleophosmin) fusion product by fluorescence in situ hybridization is an important feature for PACLCL to be distinguished from SALCL. However, because immunohistochemical staining for ALK is so specific and sensitive, it gradually replaces the molecular genetic studies for diagnostic purposes.

Comparative genomic hybridization (CGH) analysis demonstrated gains on chromosomes 7q and 17q and losses on 6q and 13q in PCALCL cases (27). These cases also showed higher expression of the skin-homing chemokine receptor genes CCR10 and CCR8, as compared to primary cutaneous peripheral T-cell lymphoma not otherwise specified (27). Another study of PCALCL with CGH also showed chromosomal imbalances with gains of several putative oncogenes, such as FGFR1(8p11), NRAS(1p13.2), MYCN(2p24.1), RAF1(3p25), CTSB(8p22), FES(15q26.1), and CBFA2(21q22.3) (28).

Immunohistochemistry and immunofluorescence studies revealed that PCALCL had higher expression of Notch1 and Jagged1 as compared with LyP (29). These findings imply a potential role for the Notch signaling pathway in

TABLE 6.46.2

Salient Features for Laboratory Diagnosis of PCALCL

1. CD30 is positive in >75% of tumor cells.
2. ALK and EMA are negative.
3. Clusterin is positive in about 50% of cases.
4. CLA is present.
5. Most cases are of T-helper cell subtype (CD4+, CD8-), but rare cases can be CD4-, CD8-, or CD4-, CD8+.
6. Cytotoxic proteins (TIA-1, granzyme B and perforin) are positive in most cases.
7. Selective loss of T-cell markers (CD2, CD3, or CD5) often occurs.
8. CCR4 is positive.
9. EBV is rarely positive in extracutaneous disseminated cases.
10. T-cell receptor gene rearrangement is monoclonal in most cases.
11. t(2;5) translocation is not present.

ALK, anaplastic lymphoma kinase; CCR4, CC-chemokine receptor 4; EMA, epithelial membrane antigen; PCALCL, primary cutaneous anaplastic large-cell lymphoma, TIA-1, T-cell intracellular antigen-1.

the pathogenesis of both diseases and provide a rationale for the use of Notch antagonists in the treatment of these diseases.

The current case shows a cutaneous anaplastic large-cell lymphoma with an immunophenotype of CD3+, CD30+, ALK-, CD20-, CD15-, and no clinical evidence of systemic disease, thus excluding SALCL and in favor of PCALCL. The rapid transformation in clinical course and the presence of constitutional symptoms rule out LyP. The location of lesions in the leg raises the question of primary cutaneous diffuse large B-cell lymphoma, leg type, but the immunophenotyping excludes this diagnosis. Finally, examination of the previous skin biopsy revealed that the old dermatitis was due to psoriasis and not MF, thus excluding the tumor stage of MF. The salient features for laboratory diagnosis of PCALCL are summarized in Table 6.46.2.

Clinical Manifestation

PCALCL mainly involves elderly people with a medium age of 60 years (2). Children, on the other hand, have a high incidence of SALCL but rarely PCALCL (17). Unlike SALCL, there is no gender preference in PCALCL. The skin lesions are frequently located in the trunk, face, extremities, and buttocks (2). In the neutrophil/eosinophil-rich variant, trunk and head are more frequently involved (9,10). These lesions are usually presented as a rapidly growing, solitary ulcerating tumor mass, but papules can be seen in some cases (1,2). Multifocal lesions are found in about 20% of patients (1,2). The neutrophil/eosinophil

variant is characterized by an ulcerative, purulent lesion, but erythematous painful nodules and keratoacanthoma-like lesions have also been described (7–9). Similar to LyP, PCALCL lesions may have partial or complete regression, but relapse is commonly seen. Extracutaneous dissemination seldom occurs. If present, it usually involves the regional lymph nodes. Surgical excision or localized radiotherapy is usually sufficient for the treatment of PCALCL. On the other hand, systemic chemotherapy is necessary for the management of SALCL. The prognosis of PCALCL is generally more favorable than SALCL with a 10-year disease-related survival of about 90% (2).

REFERENCES

1. Willemze R, Jaffe ES, Burg G, et al. WHO-EORTC classification for cutaneous lymphoma. *Blood*. 2005;105:3768–3785.
2. Ralfkiaer E, Willemze R, Paulli M, et al. Primary cutaneous CD30-positive T-cell lymphoproliferative disorders. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:300–301.
3. Kadin ME, Carpenter C. Systemic and primary cutaneous anaplastic large cell lymphomas. *Sem Hematol*. 2003;40:244–256.
4. Kinney MC, Collins RD, Greer JP, et al. A small-cell-predominant variant of primary Ki-1 (CD30)+ T-cell lymphoma. *Am J Surg Pathol*. 1993;17:859–868.
5. Kamita T, Saga K, Yanagisawa K, et al. Small cell variant of CD30+ primary cutaneous T-cell lymphoma with epidermotropism that completely regressed after incisional skin biopsy. *Br J Dermatol*. 2006;155:477–500.
6. Kato N, Mizuno O, Ito K, et al. Neutrophil-rich anaplastic large cell lymphoma presenting in the skin. *Am J Dermatopathol*. 2003;25:142–147.
7. Lin JH, Lee JY. Primary cutaneous CD30 anaplastic large cell lymphoma with keratoacanthoma-like pseudocarcinomatous hyperplasia and marked eosinophilia and neutrophilia. *J Cutan Pathol*. 2004;31:458–461.
8. Salama S. Primary “cutaneous” T-cell anaplastic large cell lymphoma, neutrophil-rich variant with subcutaneous panniculitic lesions, in a post-renal transplant patient: report of unusual case and literature review. *Am J Dermatopathol*. 2005;27:217–223.
9. Kong YY, Dai B, Kong JC, et al. Neutrophil/eosinophil-rich type of primary cutaneous anaplastic large cell lymphoma: a clinicopathological, immunophenotypic and molecular study of nine cases. *Histopathology*. 2009;55:189–196.
10. Burg G, Kempf W, Kazakov DV, et al. Pyogenic lymphoma of the skin: a peculiar variant of primary cutaneous neutrophil-rich CD30+ anaplastic large cell lymphoma. Clinicopathological study of four cases and review of the literature. *Br J Dermatol*. 2003;148:580–586.
11. Ezra N, van Dyke GS, Binder SW. CD30 positive anaplastic large-cell lymphoma mimicking Langerhans cell histiocytosis. *J Cutan Pathol*. 2010;37:787–792.
12. Cepeda LT, Pieretti M, Chapman SP, et al. CD30-positive atypical lymphoid cells in common non-neoplastic cutaneous infiltrates rich in neutrophils and eosinophils. *Am J Surg Pathol*. 2003;27:912–918.
13. Kadin ME, Pinkus JL, Pinkus GS, et al. Primary cutaneous ALCL with phosphorylated/activated cytoplasmic ALK and novel phenotype: EMA/MUC1+, cutaneous lymphocyte antigen negative. *Am J Surg Pathol*. 2008;32:1421–1426.

14. Saffer H, Wahed A, Rassidakis GZ, et al. Clusterin expression in malignant lymphomas: a survey of 266 cases. *Mod Pathol*. 2002;15:1221–1226.
15. Lae ME, Ahmed I, Macon WR. Clusterin is widely expressed in systemic anaplastic large cell lymphoma but fails to differentiate primary from secondary cutaneous anaplastic large cell lymphoma. *Am J Clin Pathol*. 2002;118:773–779.
16. Olsen SH, Ma L, Schnitzer B, et al. Clusterin expression in cutaneous CD30-positive lymphoproliferative disorders and their histologic simulants. *J Cutan Pathol*. 2009;36:302–307.
17. Kumar S, Pittaluga S, Raffeld M, et al. Primary cutaneous CD30-positive anaplastic large cell lymphoma in childhood: report of 4 cases and review of the literature. *Pediatr Develop Pathol*. 2005;8:52–60.
18. ten Berge RL, Oudejans JJ, Ossenkoppele GJ, et al. ALK-negative systemic anaplastic large cell lymphoma: differential diagnostic and prognostic aspects—a review. *J Pathol*. 2003;200:4–15.
19. Magro CM, Dyrsen ME. Cutaneous lymphocyte antigen expression in benign and neoplastic cutaneous B- and T-cell lymphoid infiltrates. *J Cutan Pathol*. 2008;35:1040–1049.
20. Kempf W, Kutzner H, Cozzio A, et al. MUM1 expression in cutaneous CD30+ lymphoproliferative disorders: a valuable tool for the distinction between lymphomatoid papulosis and primary cutaneous anaplastic large-cell lymphoma. *Br J Dermatol*. 2008;158:1280–1287.
21. Wasco MJ, Fullen D, Su L, et al. The expression of MUM1 in cutaneous T-cell lymphoproliferative disorders. *Hum Pathol*. 2008;39:557–563.
22. Hernandez-Machin B, de Misa RF, Montenegro T, et al. MUM1 expression does not differentiate primary cutaneous anaplastic large-cell lymphoma and lymphomatoid papulosis. *Br J Dermatol*. 2009;160:710–724.
23. Benner MF, Habsen PM, Meijer CJLM, et al. Diagnostic and prognostic evaluation of phenotypic markers TRAF1, MUM1, BCL2 and CD15 in cutaneous CD30-positive lymphoproliferative disorders. *Br J Dermatol*. 2009;161:121–127.
24. Natkunam Y, Warnke RA, Haghighi B, et al. Coexpression of CD56 and CD30 in lymphomas with primary presentation in the skin: clinicopathologic, immunohistochemical and molecular analyses of seven cases. *J Cutan Pathol*. 2000;27:392–399.
25. Kim YC, Yang WI, Lee MG, et al. Epstein-Barr virus in CD30+ anaplastic large cell lymphoma involving the skin and lymphomatoid papulosis in South Korea. *Int J Dermatol*. 2006;45:1312–1316.
26. Greisser J, Palmedo G, Sander C, et al. Detection of clonal rearrangement of T-cell receptor genes in the diagnosis of primary cutaneous CD30+ lymphoproliferative disorders. *J Cutan Pathol*. 2006;33:711–713.
27. van Kester MS, Tensen CP, Vermeer MH, et al. Cutaneous anaplastic large cell lymphoma and peripheral T-cell lymphoma NOS show distinct chromosomal alterations and differential expression of chemokine receptors and apoptosis regulators. *J Invest Dermatol*. 2009;130:563–575.
28. Mao X, Orchard G, Lillington DM, et al. Genetic alterations in primary cutaneous CD30+ anaplastic large cell lymphoma. *Genes Chromosomes Cancer*. 2003;37:176–185.
29. Kamstrup MR, Ralfkiaer E, Skovgaard GL, et al. Potential involvement of Notch1 signalling in the pathogenesis of primary cutaneous CD30-positive lymphoproliferative disorders. *Br J Dermatol*. 2008;158:747–753.

CASE 47

Hodgkin Lymphoma

CASE HISTORY

A 63-year-old man came to the clinic for a prostate biopsy because his prostate serum antigen was elevated. During the subsequent workup, he was found to have hilar and paratracheal adenopathy by chest x-ray. Computed tomography scan further identified bulky disease in porta hepatis lymph node with compression of the stomach, multiple liver nodules, and adenopathies in the mesenteric, periaortic, and paratracheal regions. At that time, the patient also had low-grade fever, drenching night sweats, and pruritus. He had lost 30 lb in the past 6 months.

Physical examination on admission revealed hepatosplenomegaly but no superficial lymphadenopathy. His total leukocyte count was 4,500/mL, hematocrit 37%, hemoglobin 12 g/dL, and platelets 479,000/mL. The serum lactate dehydrogenase was 378 U/dL.

Because no lymph nodes were accessible, a bone marrow biopsy was done; it showed features that were suspicious of Hodgkin lymphoma (HL). The subsequent mediastinal lymph node biopsy confirmed that diagnosis. The

patient was treated with HL regimen and showed clinical improvement, including gaining 5 lb and having fewer night sweats and less skin rash.

FLOW CYTOMETRY FINDINGS

Lymph node biopsy: CD3/CD4 67%, CD3/CD8 25%, CD5 87%, CD19 11%, CD19/k 6%, CD19/l 4%, CD20 13%, CD23 8%, FMC-7 12%, CD10 1%, CD45 100%.

DISCUSSION

HL is a unique tumor in that the tumor cell is in the minority of the cellular composition (about 1%), whereas the major component in the background histology is reactive inflammatory cells (1,2). The comprehensive review by Taylor and Riley (3) describes the interesting history of longstanding efforts to elucidate the nature of the tumor cell (Reed-Sternberg [RS] cell), with various techniques,



including microbiology, serology, immunohistochemistry, cell culture, animal inoculation, and finally molecular genetics. At the beginning, HL was considered an infectious disease; when it became evident that it is a malignancy, the cell lineage of the tumor cells had been elusive for many years until recently. Only when a micromanipulative technique is applied in combination with the polymerase chain reaction (PCR) to single RS cells are the RS cells finally identified as clonal B cells. As a result, Hodgkin disease is now considered a lymphoma; thus, the name has been changed to HL (1,2).

Since the Rye Conference in 1966 (4), the classification of HL went unchanged until 1994 when the International Lymphoma Study Group updated the classification (5). The original classification divided HL into lymphocyte predominance, nodular sclerosis, mixed cellularity, and lymphocyte depletion. In the Revised European-American classification of lymphoid neoplasms, the lymphocyte predominance type is redefined to include only the nodular subtype of lymphocyte predominance. A lymphocyte-rich classical Hodgkin lymphoma (LRCHL) type has been created to include the diffuse subtype of lymphocyte predominance, some cases of cellular phase of nodular sclerosis, and some cases of mixed cellularity in the Rye classification (5). The World Health Organization (WHO) classification separates the lymphocyte predominance type from the classical HL (cHL) and designates it as nodular lymphocyte predominance Hodgkin lymphoma (NLPHL) (6,7). The remaining four types are classified as cHL (Table 6.47.1).

Recently, Eberle et al. (8) supported a three-disease hypothesis of HL suggested by MacMahon in 1966. The idea is that mixed cellularity and lymphocyte-depleted types may be part of a biologic continuum, and nodular sclerosis

is a distinct entity with different epidemiology, clinical presentation, and histology. NLPHL is further distinguished from the other two entities. The differences between these three categories are listed in Table 6.47.2 (8).

Morphology

The most important criterion for the diagnosis of HL is the identification of a small number of large mononucleated and multinucleated tumor cells, designated Hodgkin and RS cells (HRS cells) (9,10). HRS cells are characterized by their large size, bilobed or polylobated nuclei, or multinucleation, with prominent eosinophilic inclusion-like nucleoli and abundant amphophilic cytoplasm (Figs. 6.47.1 and 6.47.2). There are several variants of HRS cells. The presence of the variants alone is not diagnostic, but it should be an indication of the need to search further for diagnostic cells. The mononuclear variant is a large cell with a single nucleus that contains a prominent eosinophilic nucleolus. This cell is called a mononucleated Hodgkin cell. A cell with similar features, but multinuclear, is called a multinucleated Hodgkin cell. Mononucleated and multinucleated Hodgkin cells are not diagnostic in an unknown case, but their presence is sufficient for the diagnosis of extranodal HL in patients with known nodal HL. The degenerated form of a tumor cell is a dark smudged cell, which is called a “mummified cell.”

The other two variants are also not diagnostic of HL, but are characteristic of certain types of HL. The lymphocyte predominant (LP) cells, formerly known as lymphocytic and/or histiocytic (L&H) cell variant or popcorn cells, are large cells with folded, convoluted, or lobated nuclei; thin nuclear membrane; vesicular chromatin pattern; and inconspicuous nucleoli (Fig. 6.47.3) (7). The cytoplasm of these cells is usually abundant and pale staining. However, when LP cells are predominant showing the characteristic immunophenotype, it is diagnostic of NLPHL. In this type of HL, RS cells are not present (8). The lacunar variant is a large cell located in a lacunalike space. This phenomenon is considered to be an artifact due to formalin fixation, because the lacunalike space is not seen in other forms of fixation. The lacunar cells have monolobated or polylobated nuclei, delicate nuclear chromatin, small nucleoli, and an abundant water-clear or pale eosinophilic cytoplasm (Fig. 6.47.4).

The identification of these tumor cells in the lymph node frequently leads to a definitive diagnosis. When the tumor cells are seen in extranodal tissues, however, a diagnosis of HL cannot be established unless the patient has a history of HL diagnosed by lymph node biopsy. In the bone marrow, the characteristic feature is focal fibrosis or focal increase of cellularity (Fig. 6.47.5) (11). The cellular component usually includes eosinophils, plasma cells, histiocytes, and a few Hodgkin tumor cells, but classical RS cells are seldom present. It is important to use immunohistochemical stains to confirm the diagnosis.

Nodular Lymphocyte Predominance Hodgkin Lymphoma

This type is characterized by a vague or a partially nodular pattern with diffuse areas. Reticulin stain may accentuate

TABLE 6.47.1	
Comparison of WHO Classification and Rye Classification	
WHO classification	Rye classification
NLPHL	Lymphocyte predominance, nodular subtype
Classical HL	
NSCHL	Nodular sclerosis
MCCHL	Mixed cellularity
LRCHL*	Lymphocyte predominance, diffuse subtype
LDCHL	Lymphocyte depletion

*Includes some cases of mixed cellularity and cellular subtype of nodular sclerosis.
HL, Hodgkin lymphoma; LDCHL, lymphocyte-depleted classical Hodgkin lymphoma; LRCHL, lymphocyte-rich classical Hodgkin lymphoma; MCCHL, mixed cellularity classical Hodgkin lymphoma; NLPHL, nodular lymphocyte predominance Hodgkin lymphoma; NSCHL, nodular sclerosis classical Hodgkin lymphoma.

TABLE 6.47.2

Differences between Three Major Categories of HL

	NSCHL	MCCHL/ LDCHL	NLPHL
Age	Young adults	Children or elderly	Young adults
Gender predominance	Female	Male	Male
Socioeconomic status	High	Low	No risk factors
EBV infection	Negative	Positive	Negative
HIV infection	Negative	Positive	
Lesion location	Mediastinal, cervical and axial lymph nodes	Generalized disease, lymph nodes, and bone marrow	Peripheral and mesenteric lymph nodes, no mediastinal involvement

EBV, Epstein-Barr virus; HIV, Human immunodeficiency virus; LDCHL, lymphocyte-depleted classical Hodgkin lymphoma; MCCHL, mixed cellularity classical Hodgkin lymphoma; NLPHL, nodular lymphocyte predominance Hodgkin lymphoma; NSCHL, nodular sclerosis classical Hodgkin lymphoma.

the nodular configuration. The background is composed of small lymphocytes, histiocytes, and epithelioid histiocytes. Eosinophils, neutrophils, plasma cells, and necrosis are rarely seen. The tumor cells are mainly LP cells, with accompanied mononuclear Hodgkin cells, mummified cells, and abnormal mitosis. However, classical RS cells are absent in this type (8). In rare cases, follicular hyperplasia with progressive transformation of germinal centers (PTGC) may be present (12). These germinal centers contain various proportions of small lymphocytes of mantle zone type (Fig. 6.47.6). This lesion may be similar to T-cell-rich B-cell lymphoma, but the latter does not contain nodular pattern.

Nodular Sclerosis Classical Hodgkin Lymphoma

The characteristic histologic pattern of nodular sclerosis classical HL (NSCHL) is the presence of interconnecting bands of collagen fibers separating lymphoid tissue into

cellular nodules (Fig. 6.47.7). The collagen bands can be identified by their birefringent character when examined under polarized light. The lymph node capsule may also become thickened. The characteristic tumor cells are the lacunar cells, which have subtle differences in number and in morphology among various subtypes. The number of classical RS cells also varies in different subtypes of NSCHL. The background cellular components may be predominantly lymphocytes or mixed populations of lymphocytes, eosinophils, and neutrophils. Plasma cells and histiocytes may also be present. When eosinophils account for >5% of the background cells, a poor prognosis is expected (13). On the basis of the cellular background of the nodules, NSCHL can be further divided into subtypes of lymphocytic predominance, mixed cellularity, and lymphocyte depletion. A syncytial variant of NSCHL is defined by the presence of large aggregates or sheets of lacunar cells. Focal necrosis is common in NSCHL. A two-grade system of NSCHL was

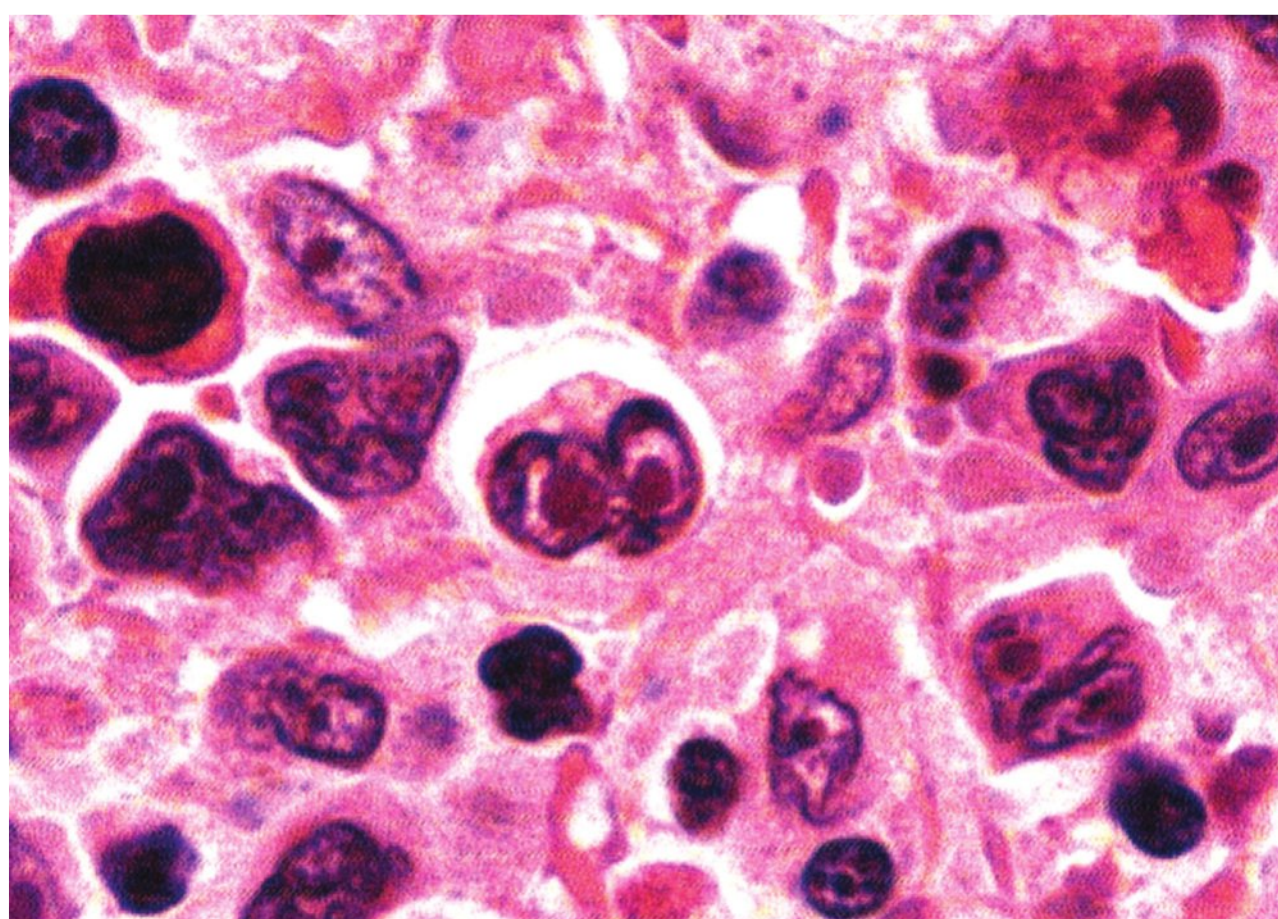


FIGURE 6.47.1 Lymph node biopsy shows a Reed-Sternberg cell in the center, surrounded by a few mononuclear and multinuclear Hodgkin cells. Hematoxylin and eosin, 200× magnification.

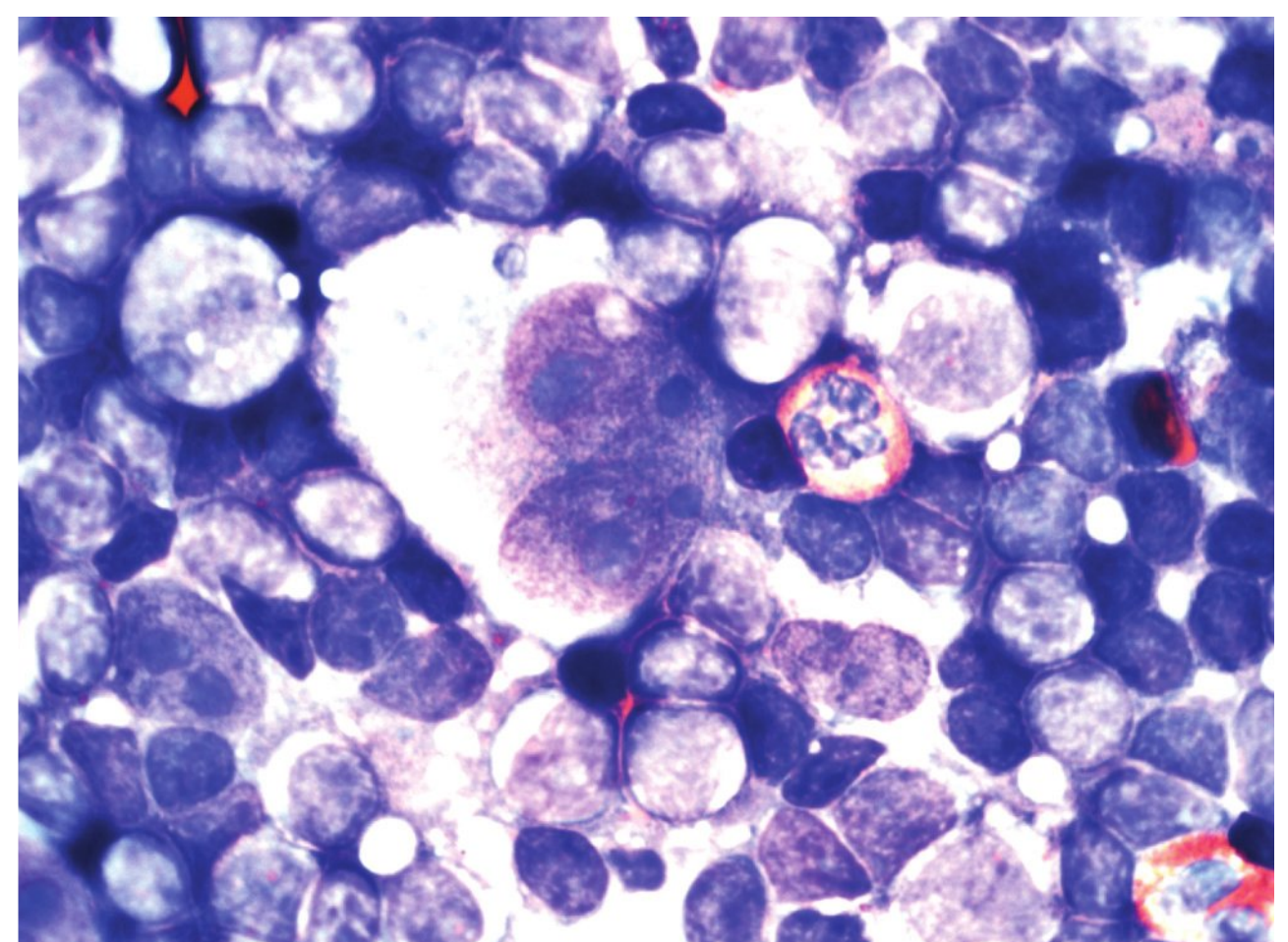


FIGURE 6.47.2 Lymph node imprint demonstrates an RS cell in the center, surrounded by lymphocytes, granulocytes, and a few eosinophils. Wright-Giemsa, 200× magnification.

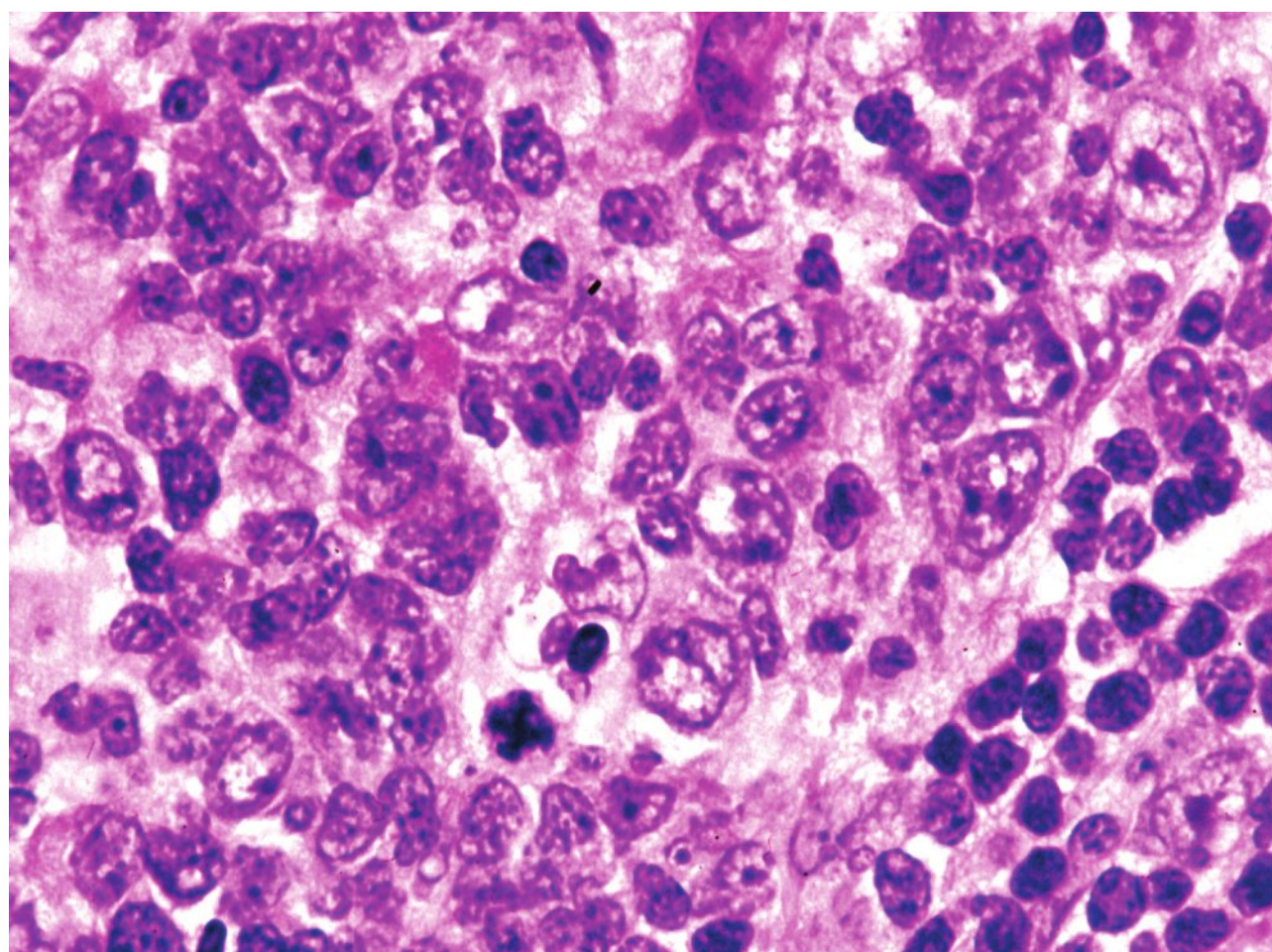


FIGURE 6.47.3 Lymph node biopsy reveals a cluster of lymphocyte predominant (LP) cells with folded, convoluted, or lobated nuclei; thin nuclear membrane, vesicular chromatin pattern, and inconspicuous nucleoli. Hematoxylin and eosin, 100× magnification.

proposed by the British National Lymphoma Investigation group based on the cellularity of the nodules, the quantity of sclerosis, and the amount and atypia of neoplastic cells (8). A new grading system is based on three criteria: eosinophilia, lymphocyte depletion, and atypia of the RS cells (14). This grading system was reported to have a significant correlation with the prognosis.

Mixed Cellularity Classical Hodgkin Lymphoma

This type was considered to be a wastebasket of unclassifiable cases in the Rye classification, but is now recognized as a true subtype of HL. It is characterized by diffuse mixed cellular infiltration with easily identifiable RS cells but without a nodular sclerosing pattern (Fig. 6.47.8). The number of classical RS cells and mature lymphocytes in

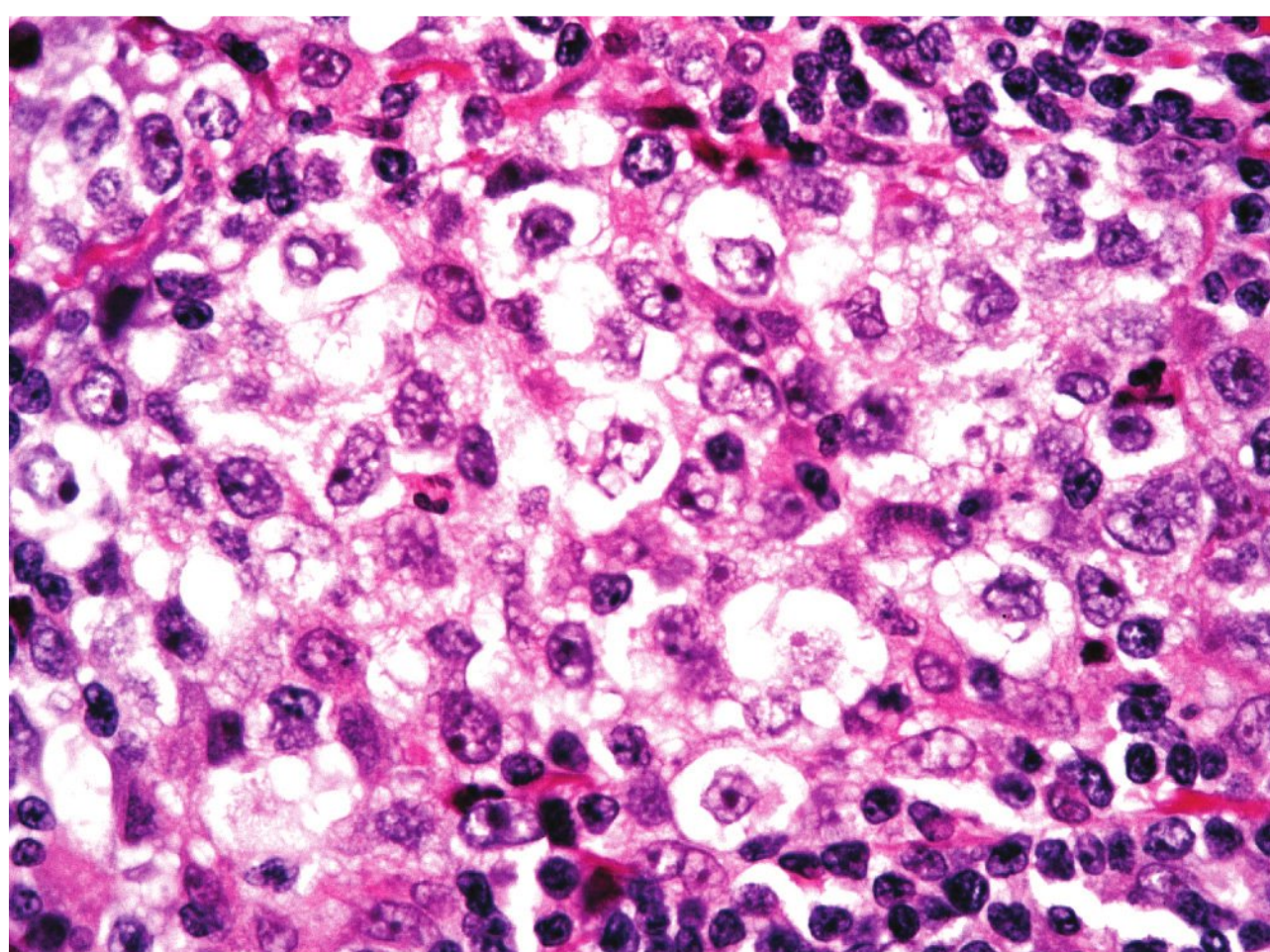


FIGURE 6.47.4 Syncytial variant of nodular sclerosis shows sheets of lacunar cells. Hematoxylin and eosin, 60× magnification.

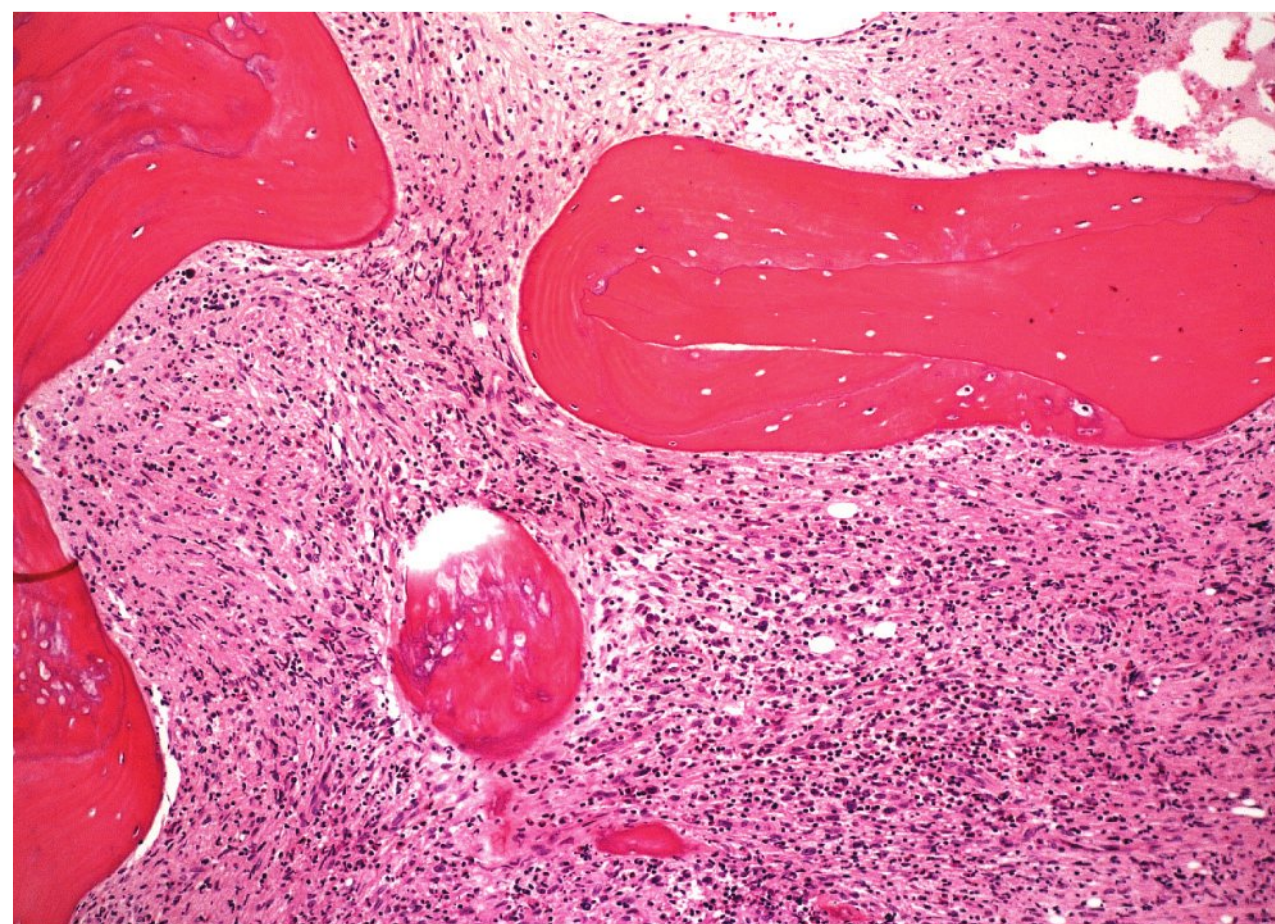


FIGURE 6.47.5 Bone marrow biopsy reveals a cellular area with fibrosis. There are scattered tumor cells with an eosinophilic background. The diagnosis of HL involvement is proved by immunohistochemistry. Hematoxylin and eosin, 10× magnification.

mixed cellularity classical HL (MCCHL) falls between that of lymphocyte-rich classical HL (LRCHL) and lymphocyte-depleted classical HL (LDCHL) (9). The background cells include lymphocytes, eosinophils, neutrophils, histiocytes, and plasma cells, but one of the cell types may be more prominent. When the epithelioid histiocytes are predominant, granuloma-like clusters may be present.

Lymphocyte-Rich Classical Hodgkin Lymphoma

In this type, the growth pattern can be nodular or diffuse. In the nodular subtype, the nodule is composed mainly of small lymphocytes and may contain regressed germinal centers. Eosinophils and neutrophils are seldom seen. The HRS cells are usually present in the expanded mantle zone. They are mostly LP cells or mononuclear lacunar cells.

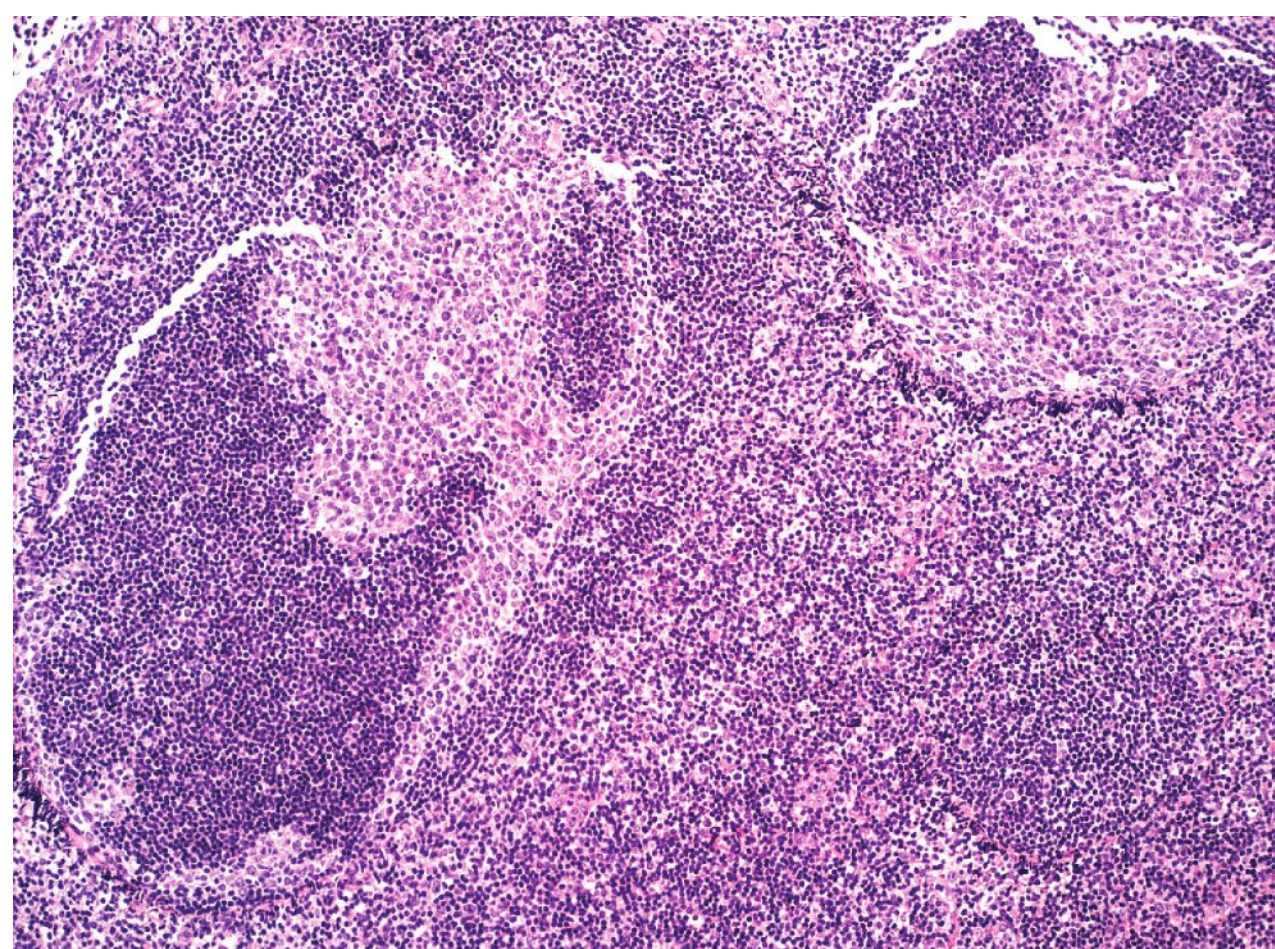


FIGURE 6.47.6 Hyperplastic lymph node shows small lymphocytic infiltration in two germinal centers, consistent with PTGC. Hematoxylin and eosin, 10× magnification.

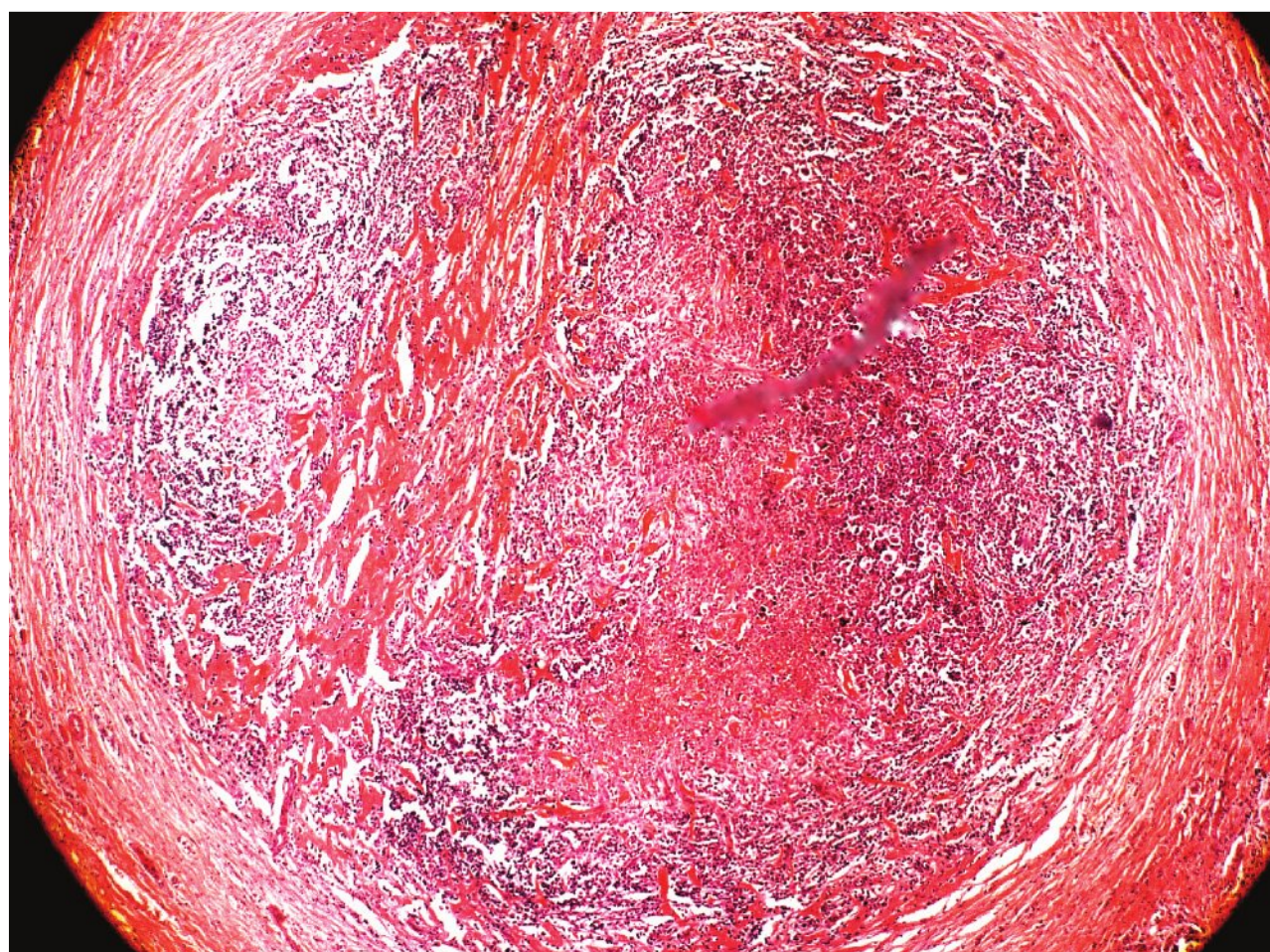


FIGURE 6.47.7 Lymph node biopsy in a case of nodular sclerosis HL reveals a cellular nodule surrounded by a thick band of collagen fibers. There is focal necrosis in the center, and scattered lacunar cells are barely recognizable. Hematoxylin and eosin, 4× magnification.

In the diffuse subtype, histiocytes and epithelioid histiocytes may be abundant (Fig. 6.47.9). Sometimes, LRCHL can only be distinguished from NLPHL by immunophenotyping (15).

Lymphocyte-Depleted Classical Hodgkin Lymphoma

LDCHL is considered to be the last histologic phase of HL, in which tumor cells predominate and reactive lymphocytes diminish (6). Thus, the classical RS and Hodgkin cells become abundant and lymphocytes are depleted along with fibrosis. On the basis of variations in RS cells and the fibrotic pattern, LDCHL is subdivided into the reticular and the diffuse fibrous subtypes.

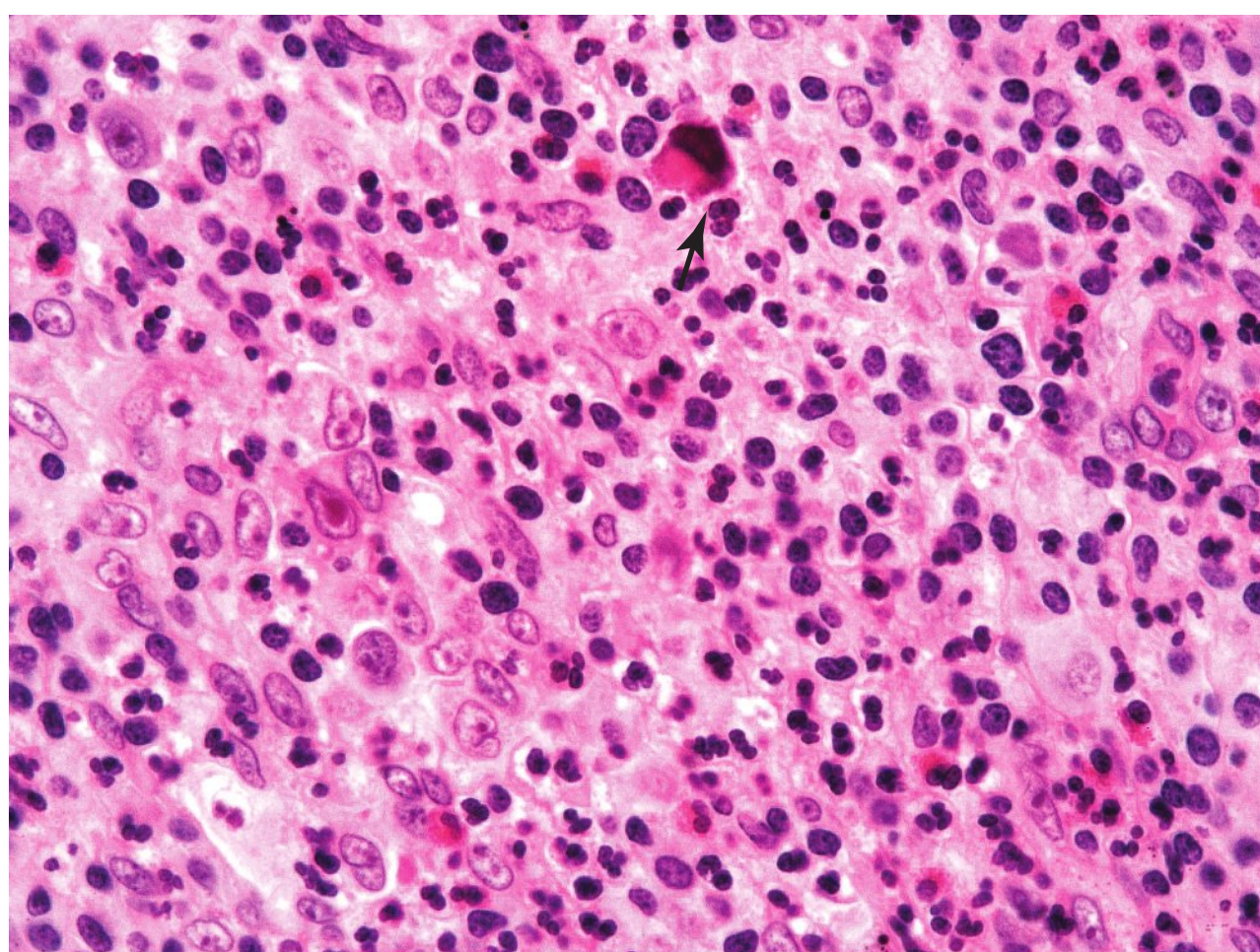


FIGURE 6.47.8 Lymph node biopsy in a case of mixed cellularity HL shows a background of lymphocytes, eosinophils, neutrophils, and histiocytes. A few Hodgkin cells and a mummified cell (arrow) are present. Hematoxylin and eosin, 60× magnification.

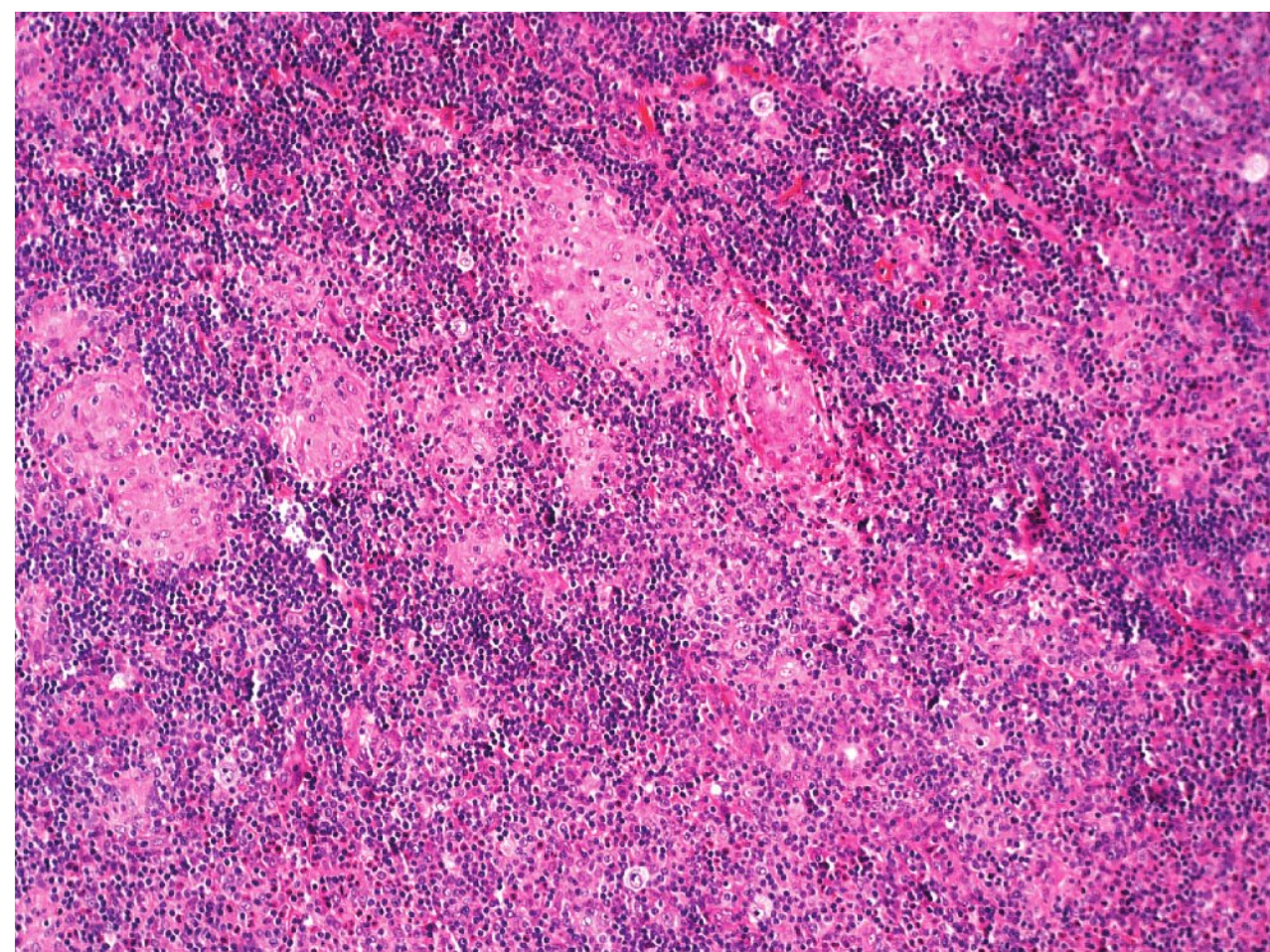


FIGURE 6.47.9 A case of diffuse subtype of lymphocyte-rich HL shows several clusters of epithelioid histiocytes mimicking granulomas. Hematoxylin and eosin, 10× magnification.

The reticular subtype is characterized by the presence of abundant classical RS cells or of bizarre multinucleated RS cells, referred to as sarcomatous RS cells (Fig. 6.47.10). In this variant, there are occasional mononuclear Hodgkin cells, mummified cells, and scattered aberrant mitoses. In the background, lymphocytes are scarce, and plasma cells, eosinophils, histiocytes, and neutrophils are also rare or absent. The fibrosis is diffuse, patchy, irregular, and fibrillar in nature. The fibrous tissue is not birefringent and thus not collagenous. Focal necrosis is common in this subtype. Many cases previously diagnosed as this subtype may well be non-Hodgkin lymphoma (NHL), particularly anaplastic large-cell lymphoma. In the subtype of diffuse

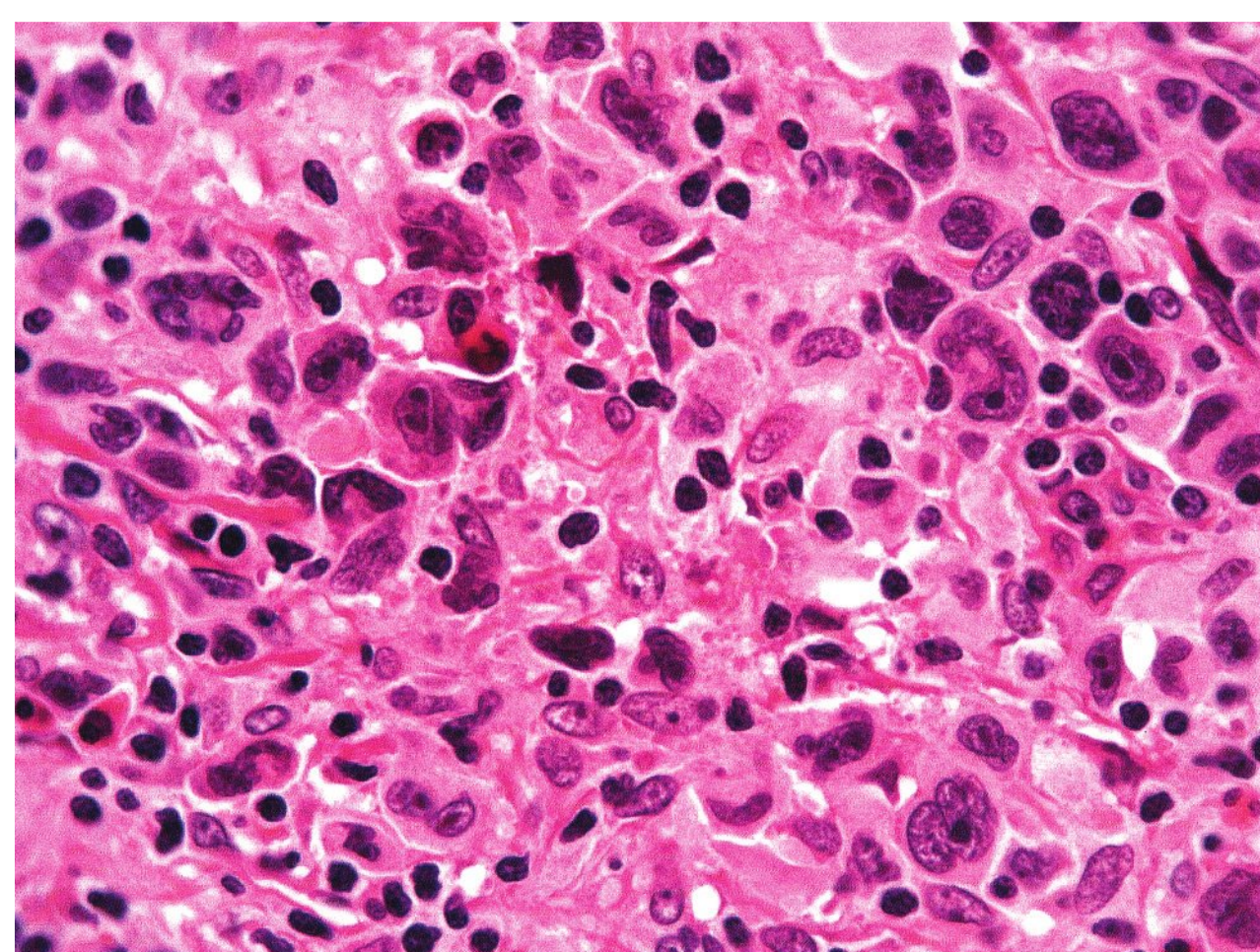


FIGURE 6.47.10 Lymph node biopsy in a case of lymphocyte-depleted HL shows multiple bizarre multinucleated HRS cells assuming a sarcomatous feature. The background contains very few lymphocytes, eosinophils, and neutrophils. Hematoxylin and eosin, 60× magnification.

fibrosis, lymphocytes are depleted, fibrosis is diffuse and disorderly, the number of RS cells is variable, and focal necrosis is frequently present. If a nodular sclerosing pattern is present, it should be classified as NSCHL.

Differential Diagnosis

Several NHLs mimic HL and are called the gray zone lymphomas, and one benign condition should also be distinguished from HL (16).

The major differential diagnosis is T-cell/histiocyte-rich B-cell lymphoma (TCRBCL), because the large lymphoma cells mimic HRS cells, whereas the background cells are normal mature T lymphocytes and histiocytes, similar to those seen in HL. Their distinction depends on immunophenotyping (see Table 6.33.1) (1,2,16).

Another lymphoma that is sometimes difficult to distinguish from HL is ALCL. The tumor cells may resemble mononuclear or multinucleated HRS cells. However, the ALCL cells frequently form cohesive sheets and involve lymph node sinuses, features that are not seen in HL (1,2,16). HL can transform into ALCL, and an intermediate form between ALCL and HL has been reported (17).

When HL is present in the mediastinum, it should be distinguished from primary mediastinal B-cell lymphoma. The latter may show a nodular sclerosing pattern with the presence of RS-like cells and occasionally an immunophenotype of CD30+, CD15+ (18).

In PTGC, the follicles may, in some cases, resemble the nodules in NLPHL. These two entities may coexist, and PTGC may occasionally develop into NLPHL; however, PTGC usually involves only a small number of lymphoid follicles. In addition, in PTGC, the normal architecture is well preserved in most parts of the lymph node, whereas in NLPHL, the entire nodal architecture is effaced (19). The major difference between these two entities is that the nodules of NLPHL contain LP cells, whereas the follicles in PTGC contain follicular center cell aggregates (20).

Immunophenotype

Because the HRS cells in HL cases are usually <1% of the cellular components, flow cytometry offers no help in identifying HRS cells and in diagnosing HL. The role of flow cytometry in HL is to exclude the possibility of NHLs, such as diffuse large B-cell lymphoma and some cases of TCRBCL (when the number of neoplastic B lymphocytes is high enough for clonal identification). In addition, flow cytometry also can identify the T-cell background in cHL, which usually shows a predominance of CD4 cells.

Immunohistochemistry, in contrast, plays a very important role in the diagnosis and classification of HL. The minimal panel for immunophenotyping includes CD30, CD15, and CD45 (Figs. 6.47.11–6.47.13). In cHL, the HRS cells are positive for CD30 and CD15, but negative for CD45. B-cell lymphomas, in contrast, should be negative for CD30 and CD15 but positive for CD45. However, some RS-like large cells in B-cell lymphomas may pick up CD30 staining, but they are usually CD15 negative.

In NLPHL, the LP cells have an immunophenotype similar to that of NHL, such as negative for CD30 and CD15 but positive for CD45 (Fig. 6.47.14) and other B-cell-associated

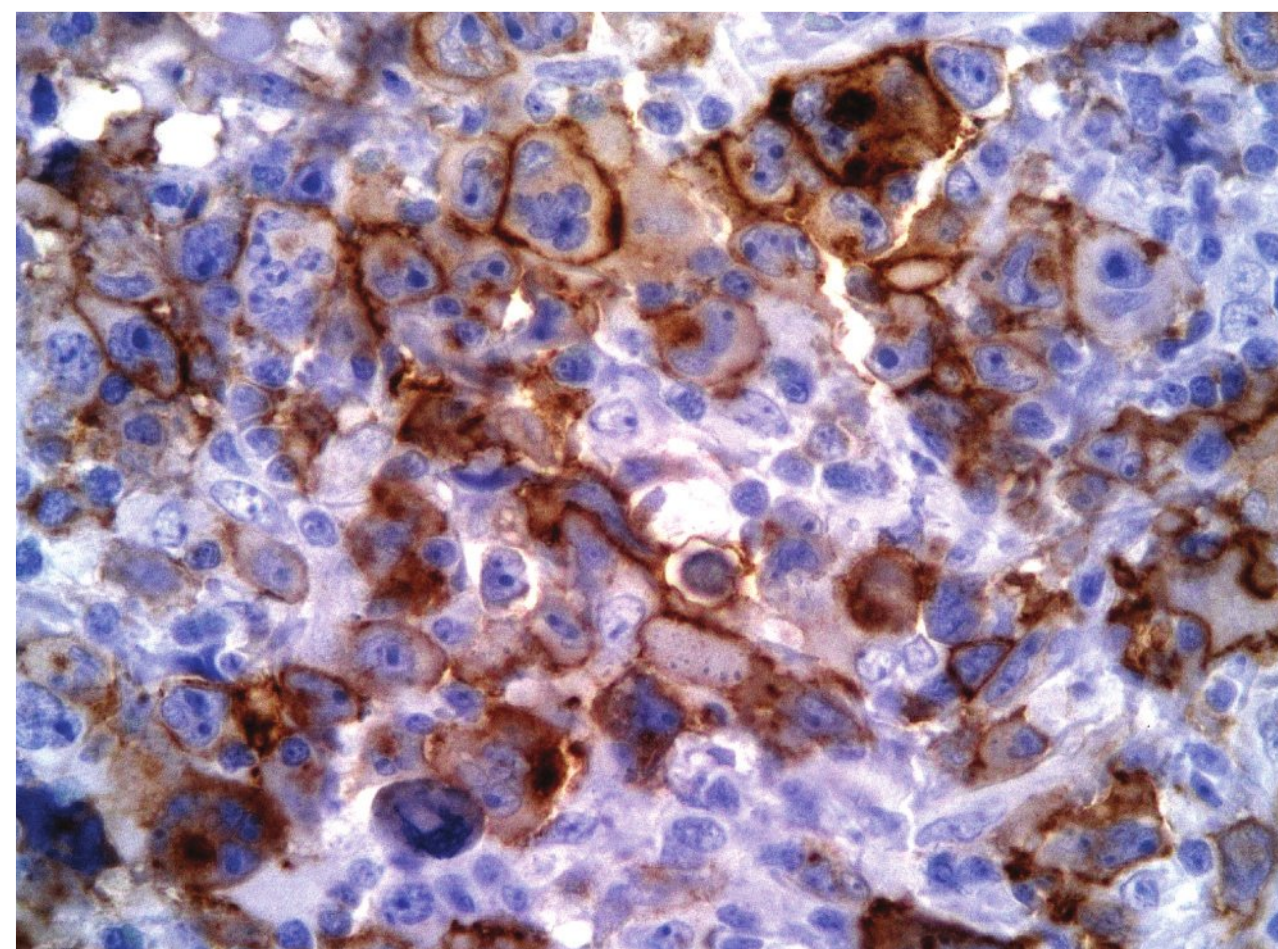


FIGURE 6.47.11 Lymph node biopsy of the syncytial variant of nodular sclerosis type shows multiple HRS cells stained with CD30. Note the membrane and Golgi staining pattern. Immunoperoxidase, 60× magnification.

antigens (CD20, CD79a, CD75) (7). Unlike B-cell lymphoma, NLPHL is positive for epithelial membrane antigen (EMA) and is generally negative for immunoglobulin (Ig) light chains (1,2), although one group reported light-chain restriction in the LP cells (21). However, J chain has been demonstrated in many cases (1,2), and clonal light-chain messenger RNA has been detected by the in situ hybridization technique (22). Furthermore, the bcl-6 protein and two activation-associated molecules, CD40 and CD86, are also expressed by the LP cells (1,2).

There are four B-cell related transcription factors (BOB1, OCT2, PU.1, and PAX5) that are important in the differential diagnosis of NLPHL. NLPHL is positive for all four factors (Fig. 6.47.15), and TCRBCL is positive for all but PU.1 (8,23). In cHL, PAX5 is positive, PU.1 is consistently absent, and BOB1 and OCT2 are rarely demonstrated in

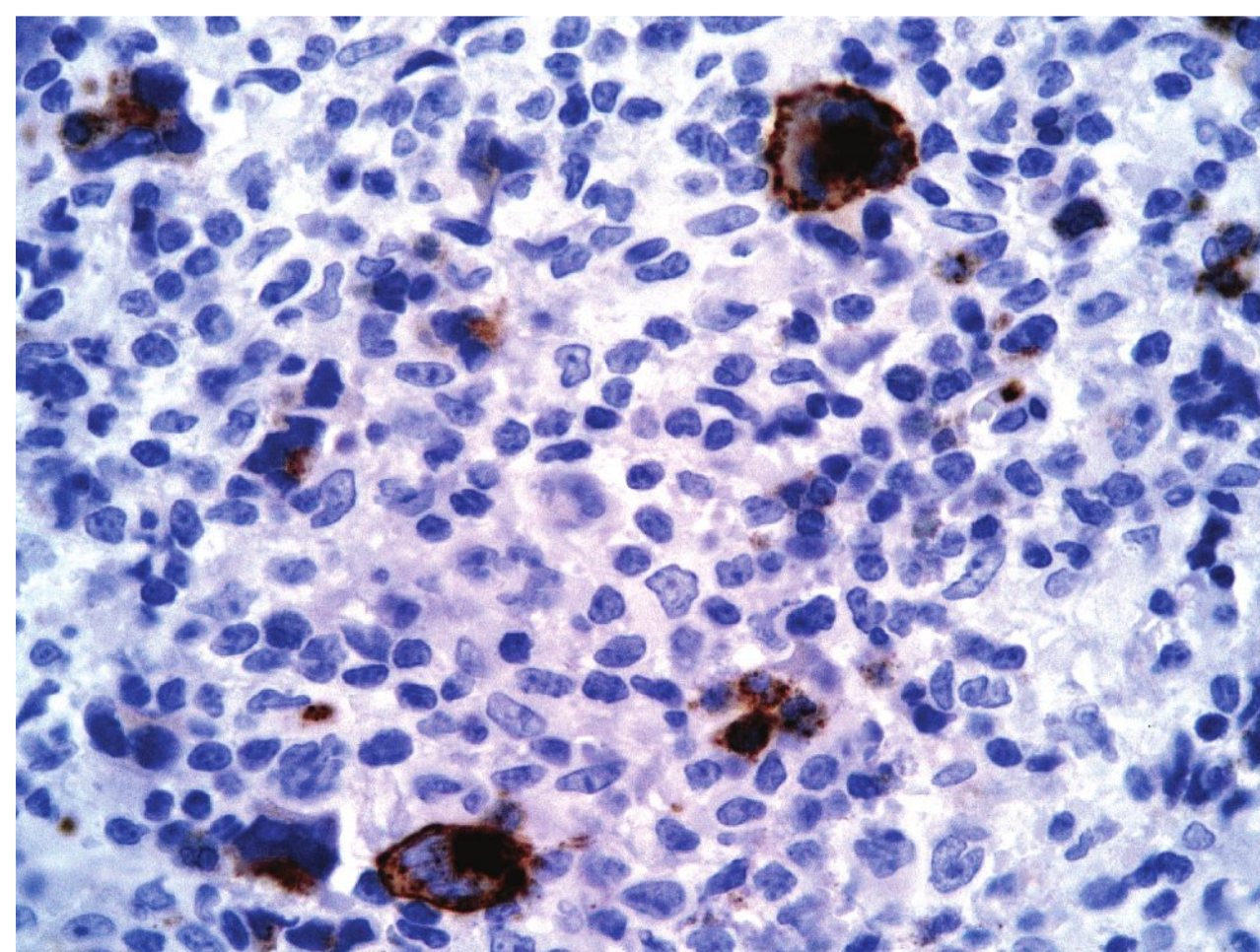


FIGURE 6.47.12 Lymph node biopsy of the same case as in Figure 6.47.11 shows two HRS cells with CD15 stain. Immunoperoxidase, 60× magnification.

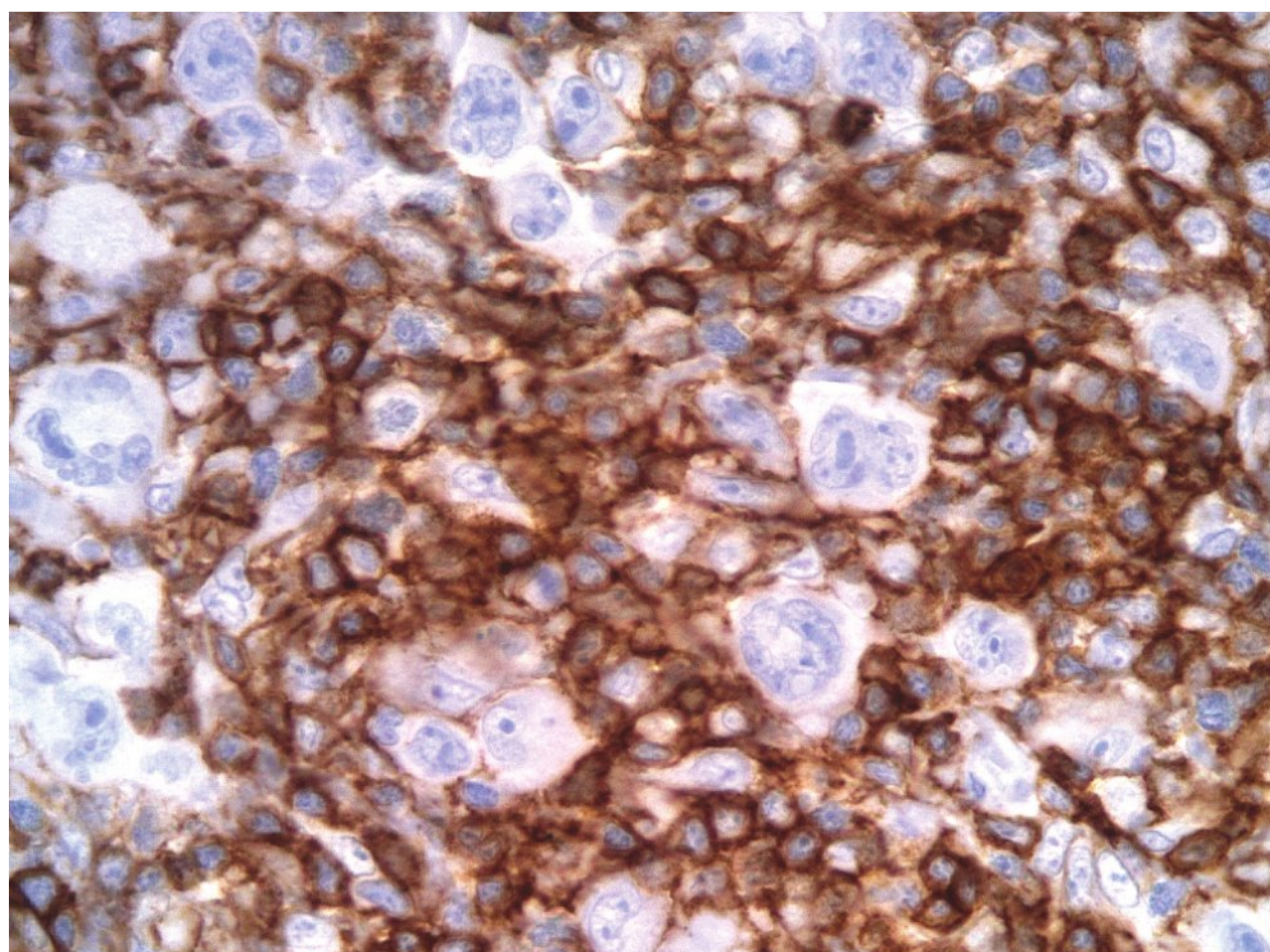


FIGURE 6.47.13 Lymph node biopsy of the same case as in Figure 6.47.11 shows CD3 staining of the background lymphocytes. The HRS cells are negative for CD3 staining. Immunoperoxidase, 60× magnification.

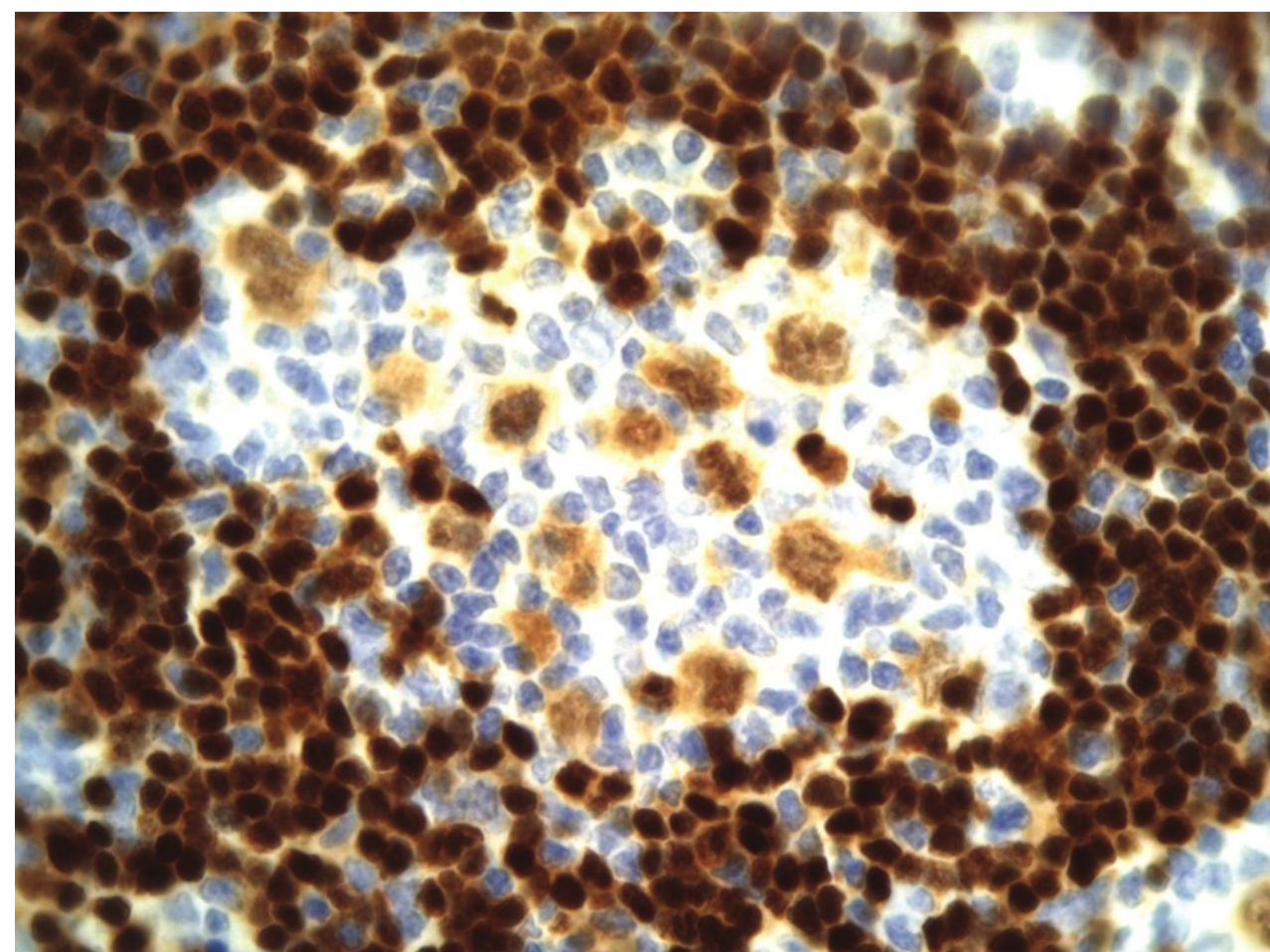


FIGURE 6.47.15 Lymph node biopsy of the same case as Figure 6.47.14 shows PAX5 staining of the LP cells. Note the LP cells stain lighter than the surrounding background B lymphocytes. Immunoperoxidase, 60× magnification.

RS cells (7). Activation-induced diaminase is also consistently demonstrated in NLPHL (7).

The small lymphocytes in the NLPHL nodules are a mixture of polyclonal B cells and T-cells. Many of the T-cells bear CD57 similar to those seen in the normal germinal centers. Some of the T-cells also coexpress CD57 and bcl-6 (24). The CD3- and the CD57-positive T cells are characterized by forming small aggregates and rosetting around the neoplastic B cells (Figs. 6.47.16 and 6.47.17). This feature distinguishes NLPHL from cHL and TCRBCL, which do not show CD57+ cell resetting (10,25). The nodules also contain meshwork of follicular dendritic cells demonstrated by CD21 or CD35 (1,2).

In the LRCHL, the HRS cells show the typical immunophenotype of CD30+, CD15+, CD45-. B-cell antigens are usually not expressed on the HRS cells, but 3% to 5% of LRCHL

cases show positive CD20 (1,2). Weak expression of one or more T-cell antigens is encountered in a minority of cases (26). The cellular nodule in LRCHL cases may show extensive CD20 staining (Fig. 6.47.18) and the follicular dendritic cell meshwork as highlighted by CD21 staining (12).

In other cHLs, including NSCHL, MCCHL, and LDCHL, the HRS cells show an immunophenotype similar to that mentioned in the LRCLH type, namely, CD30+, CD15+, CD45-, with CD20 demonstrated in 5% of cases (27). In a few studies, CD20 was demonstrated in 10% to 20% of cases in paraffin material and in 87% of cases in frozen sections (10). CD20 staining is usually variable in intensity and present only on a minority of the tumor cells (28,29). CD79a is seldom present. The B-cell-specific activation protein (BSAP), a product of the PAX5 gene, is present in approximately 90% of cases, but is usually

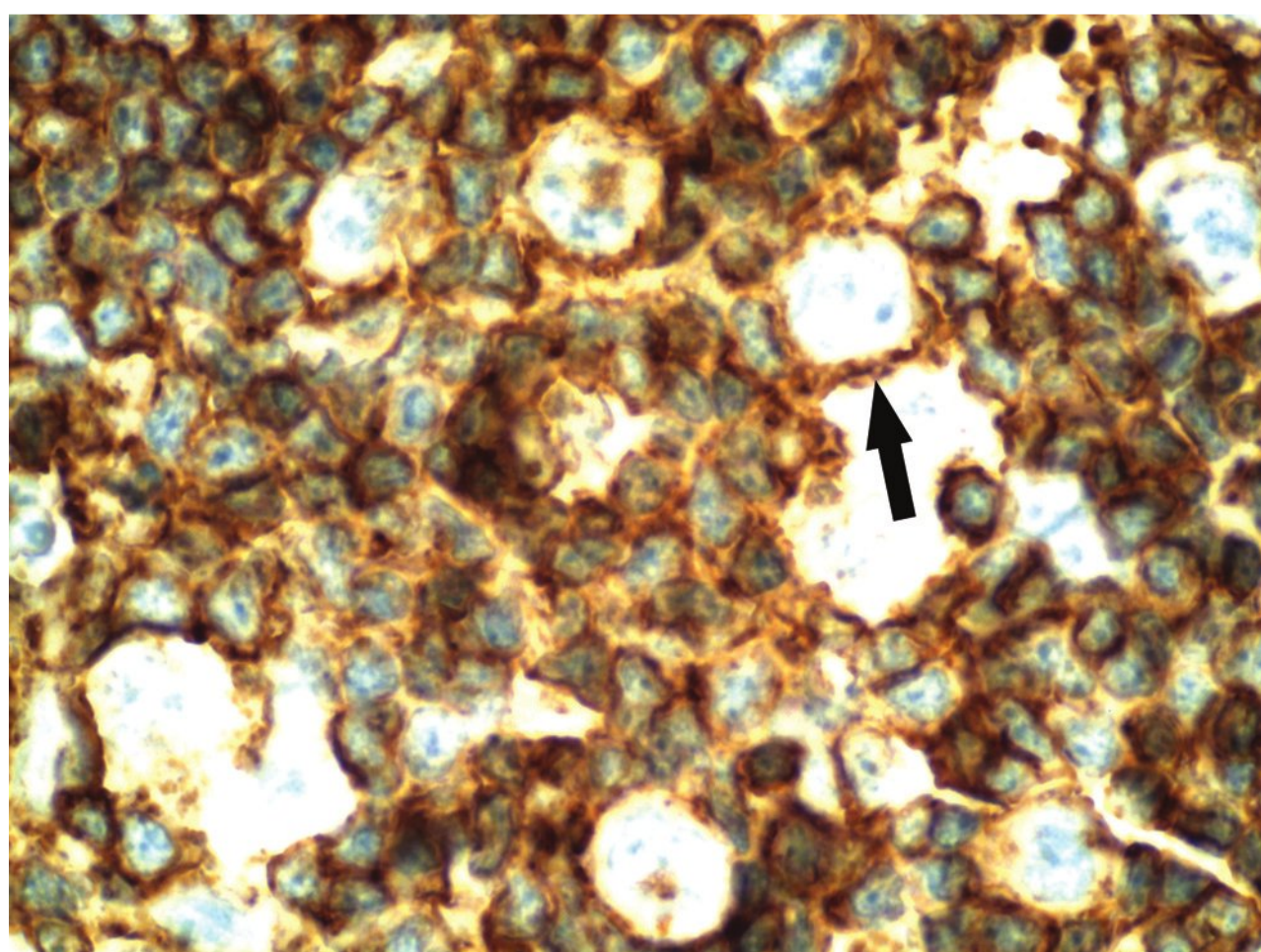


FIGURE 6.47.14 Lymph node biopsy of NLPHL shows positive CD45 staining of LP cells (arrow). Immunoperoxidase, 100× magnification.

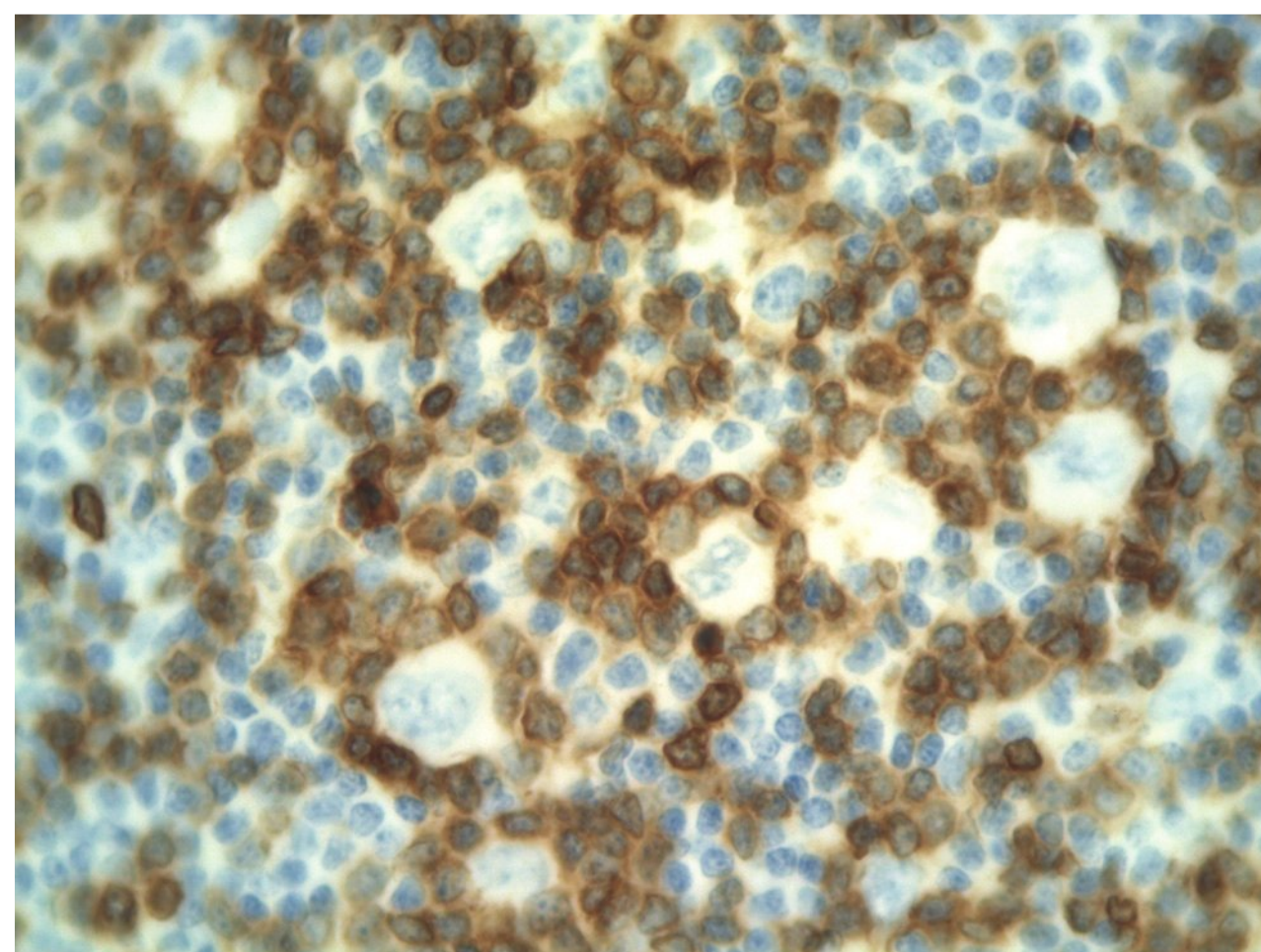


FIGURE 6.47.16 Lymph node biopsy of the same case as in Figure 6.47.14 shows CD3+ cells forming rosettes around the LP cells. Immunoperoxidase, 60× magnification.

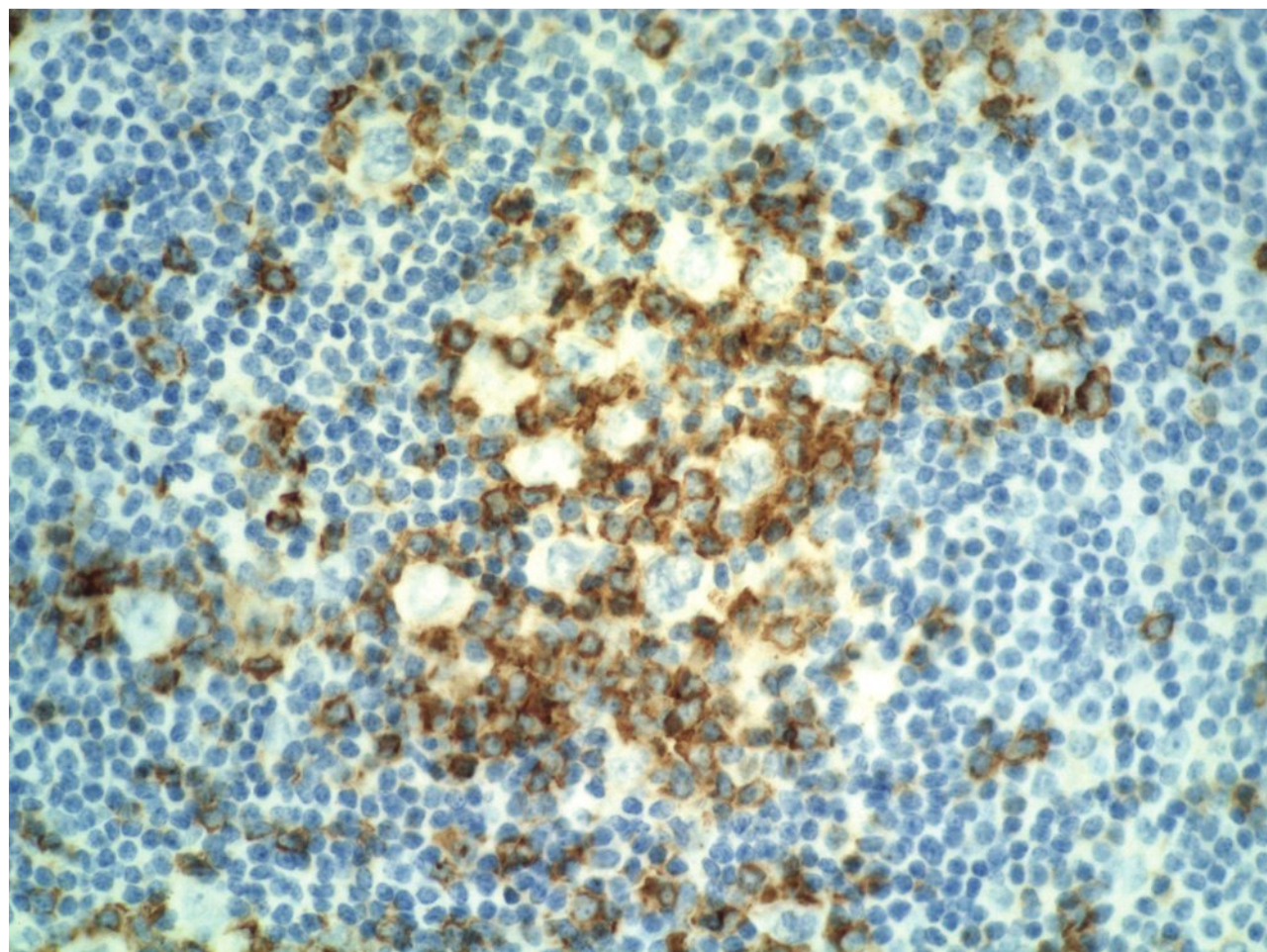


FIGURE 6.47.17 Lymph node biopsy of the same case as in Figure 6.47.14 shows CD57+ cells forming rosettes around the LP cells. Immunoperoxidase, 40× magnification.

weaker than that of reactive B cells (30). HRS cells also express IRF4/MUM1, Ki-67, CD25, HLA-DR, intercellular adhesion molecule-1, CD95, CD40, CD86, vimentin, and fascin (7,10,16). The T-cells surrounding the HRS cells are positive for CD40 ligand and CD28, the ligand for CD86 (1,2). In contrast to NLPHL, the HRS cells in cHL (except for a subset of LRCHL) cases do not express bcl-6 protein (1,2,24).

In TCRBCL, the tumor cells stain for pan-B-cell markers and are often EMA positive, but they are negative for CD30, CD15, and vimentin (1,2,31). The background lymphocytes usually show T-cell staining but are CD57 negative (16). However, a recent study showed that, in a subset of TCRBCL, the background lymphocytes were CD57 positive or coexpressed CD57 and bcl-6 (24). The large tumor

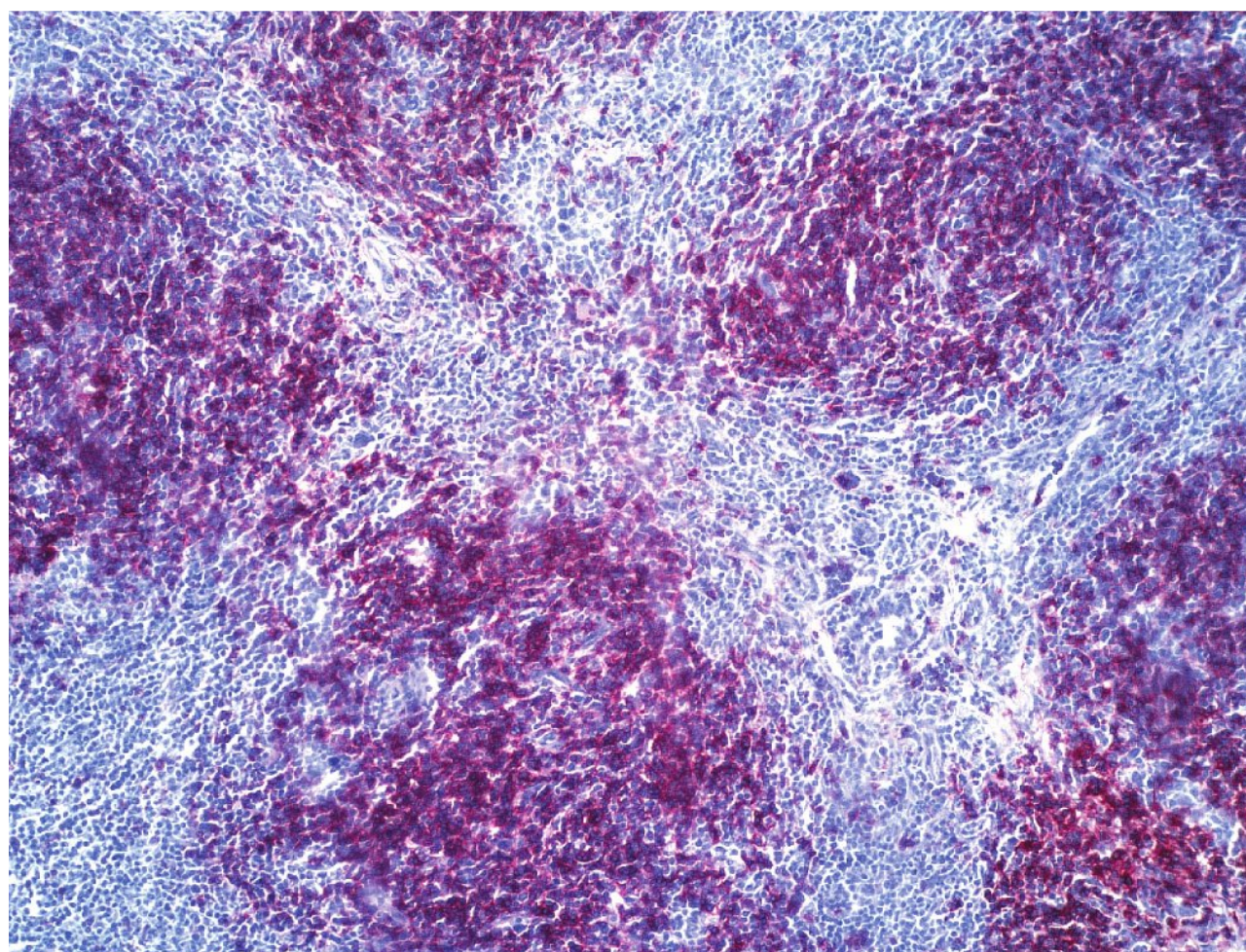


FIGURE 6.47.18 Lymph node biopsy in a case of lymphocyte-rich HL shows multiple nodules highlighted by CD20 staining. Immunoalkaline phosphatase, 10× magnification.

cells were bcl-6 positive (24). The authors suggested that this subset might be an architectural variant of NLPHL.

ALCL is usually CD30+, CD15–, EMA+. A subset of ALCL is also positive for anaplastic lymphoma kinase protein (1,2,16). Most cases of ALCL are positive for pan-T-cell markers. The CD30+ large-cell lymphoma of B-cell lineage is classified as a diffuse large B-cell lymphoma (5,6).

The immunophenotype of PTGC is similar to that of NLPHL. They both contain large nodules of B cells with follicular dendritic cell meshworks and many CD57-positive T cells (32). However, the T cells in NLPHL often form clusters, and those in PTGC are evenly distributed as in normal germinal centers. T-cell rosetting with large B cells is only seen in NLPHL (1,2).

In Epstein–Barr virus (EBV)–positive cases, the RS cells show EBV-latent membrane protein (LMP) and Epstein–Barr nuclear antigen 1 (EBNA1) but not EBNA2. EBV can be detected by showing LMP in paraffin sections, but the more sensitive technique for its identification is in situ hybridization for EBV latency–associated small RNAs (1,2). Using these methods, EBV is found in approximately 50% of cHL, but it is rarely found in NLPHL (33). In addition, some patients with HL have high titers of antibodies against EBV. Therefore, EBV may play a role in the pathogenesis of some types of HL. However, in populations in whom HL is common, the tumor cells seldom show EBV, whereas in populations in whom HL is rare, the tumor cells are frequently positive. These findings may argue against the role of EBV in the pathogenesis of HL, and EBV infection may be merely an epiphenomenon in HL (1,2).

In addition, cHL is frequently associated with overexpression of large numbers of cytokines and chemokines and/or their receptors in HRS cells (12,34). These proteins may be responsible for the presence of abundant inflammatory cells in cHL cases (12,34). For instance, the overexpression of eotaxin probably accounts for the eosinophilia in the background. Transforming growth factor- β may be responsible for the fibrosis. The CC chemokine, TARC, may contribute to the predominance of thymus and activation-regulated chemokine Th2 cells in the infiltrating T-cell population in cHL cases.

Recently, autoimmune and inflammatory conditions have been considered to play a potential role in the etiology and pathogenesis of HL (35). Family studies implicate genetic factor involvement. The increased incidence of HL in Asian immigrants in the western countries is suggestive of the important influence by environmental factors and life style (36).

Comparison of Flow Cytometry and Immunohistochemistry

The diagnosis and differential diagnosis of HL depend on immunohistochemistry. Flow cytometry plays no role in the diagnosis of HL. However, Fromm et al. used nine-color flow cytometry to study 420 cases of HL and found that this technique has 88.7% sensitivity and 100% specificity for the diagnosis of this tumor (37). The criteria they used for the diagnosis included (a) expression of CD30, CD40, and CD95; (b) increased forward and side scatter compared with normal lymphocytes; (c) lack of bright expression of CD20; (d) lack of expression of CD64; and (e) a discrete cluster in multidimensional space.

TABLE 6.47.3

Salient Features of Laboratory Diagnosis of HL

cHLs

Specific markers for HRS cells: CD30+ CD15±

Other important positive markers for HRS cells:
PAX5 (BSAP), CD40, CD95

Important negative marker for HRS cells: CD45

NLPHL

Specific markers for LP cells: CD45+ CD20+ CD79a+

Other important positive markers for LP cells:
PAX5+, BOB1+, OCT2+, PU.1+, BCL-6+

Important negative markers for LP cells: CD30,
CD15

Immunophenotype for background lymphocytes

cHL: Polyclonal CD3+ CD4+ cells

NLPHL: Polyclonal B cells and T-cells with CD3+
CD57+ cells

cHL, classical Hodgkin lymphoma; HRS, Hodgkin and Reed-Sternberg; Ig, immunoglobulin; BSAP, B-cell-specific activation protein; LP, lymphocytic predominance; NLPHL, nodular lymphocyte predominant Hodgkin lymphoma.

In the current case, flow cytometry showed mainly T lymphocytes with a normal CD4/CD8 ratio. The B-lymphocyte population was in the minority and revealed no monoclonality by the k/l ratio. Thus, the flow cytometric result helped to exclude B-cell lymphoma. The diagnosis was initially made by a bone marrow biopsy, but bone marrow involvement is only seen in about 5% of HL cases and is the result of hematogenous spread. Because of bone marrow infiltration and prominent B symptoms, the patient was in stage IVb. A lymph node biopsy is necessary for the confirmation of the diagnosis of HL and for subclassification. In this case, the lymph node showed a nodular sclerosing pattern, which generally carries a favorable prognosis. However, because the patient was already in stage IVb with a bulky disease, his clinical course became rapidly progressive and he died 2 years after the diagnosis. The salient features for laboratory diagnosis of HL are summarized in Table 6.47.3.

Molecular Genetics

Monoclonality of HRS cells has never been convincingly demonstrated by immunophenotyping or by molecular biologic techniques (Southern blot and PCR) done in whole-tissue DNA. Because HRS cells are usually composed of <1% of the cellular population in HL and because polyclonal B cells may be present in the same sections, it is understandable why the old techniques failed to draw a conclusion in terms of clonality. It has been only after the use of the sophisticated single-cell micromanipulation techniques in combination with PCR that the monoclonal nature of HRS cells has been finally established (20,38,39).

The second question is the origin of the neoplastic cells. The presence of follicular dendritic cells, CD57+ T cells in the tumor nodules or in the background of NLPHL, and the expression of bcl-6 by the LP cells and some background T cells are all suggestive of the LP cell being a centroblast in the germinal center (39). The origin of the RS cells, however, is not as clearly demonstrated by immunophenotyping, but its germinal center origin is proven by molecular biologic techniques.

In all instances, sequence analysis of RS cell-derived Ig gene rearrangements demonstrated high loads of somatic mutations, which were associated with the presence of stop codons and deletions in some cases (38). Somatic mutation in the variable region of Ig heavy-chain (V_H) genes takes place in the germinal center; thus, the somatically mutated V_H genes are specific markers for germinal center B cells. Signs of typical ongoing mutations were missing in the cHL cases studied (38). However, cases of NLPHL not only showed a high load of somatic mutations but also intraclonal mutations, indicating continued mutation after establishment of the clone (20). NLPHL also shows BCL-6 translocation with various partner genes, including IgH, IKAROS, and ABP (7).

Differing from the deleterious mutations found in cHL, NLPHL does not contain mutations that would prevent their translation into functional proteins. The crippling mutations in the Ig gene in HRS cells lead to the absence of k and l gene transcripts by in situ hybridization studies (20). The absence of Ig transcripts is caused by the inactivation of the Ig promoter by the absence of octamer-dependent transcription factor (Oct2) and/or its coactivator (BOB.1) (40,41). However, B cells acquiring crippling mutations are usually efficiently eliminated within the germinal center by apoptosis; therefore, HRS cells must be rescued by some transforming events so that they can survive.

There are many possible antiapoptotic mechanisms that involve the constitutively expressed transcription factors, such as nuclear factor- κ B (NF- κ B), Stat3, Notch1, and highly expressed cFlip molecules (42–44). The involvement of NF- κ B is suggested by the fact that its inactivation restores the sensitivity of HRS cells to apoptosis in vitro (45). Most of the cHL cases express tumor necrosis factor receptor family members (CD30, CD40) and their ligands, leading to the activation of NF- κ B (34,46). Another possibility is that EBV infection induces the expression of LMP1, which possesses antiapoptotic potential by triggering BCL2 expression and acting via the CD40 cell signaling pathway, allowing cells to evade cellular apoptotic mechanisms (47,48). Activated NF- κ B is able to move into the nucleus in which it activates transcription of several target genes implicated in prevention of apoptosis (48).

In several cases of HL associated with NHL or chronic lymphocytic leukemia (either simultaneously, progressing, or subsequently), the RS cells were found by sequence analysis to share a common B-cell precursor with other B-cell neoplasms (47–51). However, the transforming event totally changes the morphology and immunophenotype of HL, whereas the transforming event for NHL or chronic lymphocytic leukemia maintains the features of the precursor B cell (38).



In addition, the rearrangements of the NHL cells (but not of the RS cells) show signs of continuing mutation (38).

HL cell line studies identify distinct gene expression profiles, which are similar to those of EBV-transformed B cells and cell lines derived from diffuse large-cell lymphomas (52).

Clonal cytogenetic abnormalities are found in most cases of cHL by conventional karyotyping, but they are not recurrent or specific (1,2). Many cases show 14q abnormalities but not t(14;18). With the fluorescence in situ hybridization technique, clonal numeric aberrations in all cases of HL are shown to fall into two groups (1,2). Comparative genomic hybridization shows recurrent gains of the chromosomal subregions on chromosomal arms 2p, 9p, and 12q and distinct high-level amplifications on chromosomal bands 4p16, 4q23–q24, and 9p23–p24 (53).

Clinical Manifestations

NLPHL is seen in approximately 5% of cases of HL (9,10). It is characteristically seen in young (<35 years of age) males. Most patients are asymptomatic. About 80% of patients are stage I or II at presentation. The most frequently involved lymph nodes are in the cervical and axillary regions, followed by the inguinal region. The prognosis of NLPHL is very favorable, but it may transform into MCCHL or LDCHL with subsequent widespread extranodal disease. NLPHL also frequently transforms into NHL, particularly diffuse large B-cell lymphoma and TCRBCL (7,54,55). Secondary malignancies, probably treatment-related, have also been reported (55). However, because 80% of untreated NLPHL patients have achieved 10-year survival, some authors suggest a reduction therapy or a watch-and-wait strategy (54).

LRCHL comprises 6% of HL (27). It is also seen predominantly in male patients with an average age higher than that of patients with NLPHL. Most patients are in stage I or II at presentation. However, mediastinal mass is more frequently encountered in LRCHL than in NLPHL but not as frequent as seen in NSCHL (1,2,54). Although relapses are frequent, the prognosis is generally good in this group of patients.

NSCHL is the most common type of HL, accounting for 60% of the total cases (9,10). This is the only type of HL with female predominance. Patients are usually <50 years of age, and 60% are stage I or II at presentation. The clinical manifestation is often cervical or supraclavicular lymphadenopathy or a mediastinal mass. NSCHL has a greater histologic stability than other types of HL. The prognosis is usually good, except for the lymphocyte depletion subtype, which is more frequently seen in men with symptoms and advanced stage of disease.

MCCHL is the second most common type of HL, accounting for approximately 30% of the total cases (9,10). This is considered to be an intermediate form between lymphocyte predominance (including NLPHL and LRCHL) and LDCHL. Therefore, the age range of patients, the clinical course, and the prognosis are all between those of lymphocyte predominance and LDCHL. The stage at presentation is usually II or III. MCCHL frequently transforms into LDCHL. It involves the abdominal lymph node and the

TABLE 6.47.4	
Ann Arbor Staging System of HL	
Stage	Degree of involvement
I	Involvement of a single lymph node region (I) or a single extralymphatic site (IE)
II	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic site (IIE)
III	Involvement of lymph node regions on both sides of the diaphragm (III) or localized involvement of an extralymphatic site (IIIE), the spleen (IIIS), or both (IIISE)
IV	Disseminated involvement of one or more extralymphatic sites with or without lymph node involvement

spleen more often, but involvement of the mediastinum is less frequent than the NSCHL type (1,2).

LDCHL is a rare type of HL seen in only 1% to 5% of total cases (9,10). It is usually encountered in elderly patients with a median age of 50 to 57 years in various reports. Male patients are predominant in this type. Most patients have constitutional symptoms, namely, night sweats in 30% of patients, fever in 60%, and weight loss in 67% (10). Patients may also have peripheral and abdominal lymphadenopathy, hepatosplenomegaly, bone marrow involvement, lymphopenia, or subdiaphragmatic disease (9). Vascular invasion and extranodal spread are common autopsy findings in these cases. About 80% of the patients are found to have stage IIIB or IVB at presentation. The prognosis is very poor, especially for patients with the reticular subtype.

In human immunodeficiency virus–infected patients, the clinical and pathologic presentations differ from those of non–acquired immunodeficiency syndrome (non-AIDS) patients. In AIDS patients, 41% to 100% have either MCCHL or LDCHL (56). Systemic B symptoms are seen in 70% to 100%, stage III or IV disease in 75% to 90%, and bone marrow involvement in 45% to 70% of AIDS patients (56). The median survival in this group of patients is approximately 18 months.

The prognosis of HL is strongly associated with the stage of the disease. The Ann Arbor classification is universally adopted (Table 6.47.4) (57). Essentially, a single lymph node region or extranodal site involvement is classified as stage I; two or more lymph node regions on the same side of the diaphragm, stage II; lymph node regions on both sides of the diaphragm, stage III; and disseminated disease, stage IV. In addition, on the basis of absence or presence of constitutional symptoms, such as fever, night sweats, and/or weight loss of 10% of body weight, the disease is further divided into substages A and B, respectively.

REFERENCES

- Harris NL. Hodgkin's disease: classification and differential diagnosis. *Mod Pathol*. 1999;12:159–176.
- Harris NL. Hodgkin's lymphomas: classification, diagnosis, and grading. *Semin Hematol*. 1999;36:220–232.
- Taylor CR, Riley CR. Molecular morphology of Hodgkin lymphoma. *Appl Immunohistochem Mol Morphol*. 2001;9:187–202.
- Lukes R, Craver L, Hall T, et al. Report of the nomenclature committee. *Cancer Res*. 1966;19:317–344.
- Harris NL, Jaffe ES, Stein H, et al. A revised European-American Classification of Lymphoid Neoplasms: a proposal from the International Lymphoma Study Group. *Blood*. 1994;84:1361–1392.
- Harris NL, Jaffe ES, Diebold J, et al. The World Health Organization Classification of Hematologic Malignancy Report of the Clinical Advisory Committee meeting. Airlie House, Virginia, November 1997. *Mod Pathol*. 2000;13:193–207.
- Stein H and 5 groups of authors: Hodgkin lymphoma. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. 4th ed. Lyon, France: IARC Press; 2008:321–334.
- Eberle FC, Mani H, Jaffe ES. Histopathology of Hodgkin's lymphoma. *Cancer J*. 2009;15:129–137.
- Grogan TM. Hodgkin's disease. In: Jaffe ES, ed. *Surgical Pathology of the Lymph Nodes and Related Organs*. 2nd ed. Philadelphia, PA: W. B. Saunders; 1995:133–192.
- Burke JS. Hodgkin's disease: histopathology and differential diagnosis. In: Knowles DM, ed. *Neoplastic Hematopathology*. 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:623–665.
- Franco V, Tripodo C, Rizzo A, et al. Bone marrow biopsy in Hodgkin's lymphoma. *Eur J Haematol*. 2004;73:149–155.
- Stein H, Delsol G, Pileri S, et al. Hodgkin lymphoma. In: Jaffe ES, Harris NL, Stein H, Vardiman JW, et al., eds. *Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2001:237–253.
- von Wasielewski R, Seth S, Franklin J, et al. Tissue eosinophilia correlates strongly with poor prognosis in nodular sclerosing Hodgkin's disease, allowing for known prognostic factors. *Blood*. 2000;95:1207–1213.
- Von Wasielewski S, Franklin J, Fischer R, et al. Nodular sclerosing Hodgkin disease: new grading predicts prognosis in intermediate and advanced stages. *Blood*. 2003;101:4063–4069.
- Anagnostopoulos I, Hansmann ML, Franssila K, et al. European Task Force on Lymphoma project on lymphocyte predominance Hodgkin disease: histologic and immunohistologic analysis of submitted cases reveals 2 types of Hodgkin disease with a nodular growth pattern and abundant lymphocytes. *Blood*. 2000;96:1889–1899.
- Rüdiger T, Jaffe ES, Delsol G, et al. Workshop report on Hodgkin's disease and related diseases ("grey zone" lymphoma). *Ann Oncol*. 1998;9(suppl 5):S31–S38.
- Mori N, Watanabe K, Yamashita Y, et al. Hodgkin's disease with subsequent transformation to CD30 positive non-Hodgkin's lymphoma in six patients. *Cancer*. 1999;85:970–979.
- Traverse-Glehen A, Pitaluga S, Gaulard P, et al. Mediastinal gray zone lymphoma. *Am J Surg Pathol*. 2005;29:1411–1421.
- Brazier RM, Oyama K. Mistaken diagnosis of Hodgkin's disease. *Hematol Oncol Clin North Am*. 1997;11:863–892.
- Chan WC. Cellular origin of nodular lymphocyte-predominant Hodgkin's lymphoma: immunophenotypic and molecular studies. *Semin Hematol*. 1999;36:242–252.
- Schmid C, Sargent C, Isaacson P. L and H cells of nodular lymphocyte-predominant Hodgkin's disease show immunoglobulin light chain restriction. *Am J Pathol*. 1991;139:1281–1289.
- Stoler M, Nichols G, Symbula M, et al. Lymphocyte-predominance Hodgkin's disease: evidence for a k light chain restriction. *Am J Pathol*. 1995;146:812–818.
- Laumen H, Nielsen PJ, Wirth T. The BOB.1/OBF.1 co-activator is essential for octamer-dependent transcription in B cells. *Eur J Immunol*. 2000;30:458–469.
- Kraus MD, Haley J. Lymphocyte predominance Hodgkin's disease: the use of bcl-6 and CD57 in diagnosis and differential diagnosis. *Am J Surg Pathol*. 2000;24:1068–1078.
- Abramson JS. T-cell/histiocyte-rich B-cell lymphoma: Biology, diagnosis, and management. *Oncologist*. 2006;11:384–392.
- Dallenbach FE, Stein H. Expression of T-cell-receptor beta chain in Reed-Sternberg cells. *Lancet*. 1989;2:828–830.
- Von Wasielewski R, Mengel M, Fischer R, et al. Classical Hodgkin's disease: clinical impact of the immunophenotype. *Am J Pathol*. 1997;151:1123–1130.
- Schmid C, Pan L, Diss T, et al. Expression of B-cell antigens by Hodgkin's and Reed-Sternberg cells. *Am J Pathol*. 1991;139:701–707.
- Zukerberg LR, Collins AB, Ferry JA, et al. Coexpression of CD15 and CD20 by Reed-Sternberg cells in Hodgkin's disease. *Am J Pathol*. 1991;139:475–483.
- Foss HD, Reusch R, Demel G, et al. Frequent expression of the B-cell-specific activator protein in Reed-Sternberg cells of classical Hodgkin's disease provides further evidence for its B-cell origin. *Blood*. 1999;94:3108–3113.
- Rüdiger T, Ott G, Ott MM, et al. Differential diagnosis between classic Hodgkin's lymphoma, T-cell-rich B-cell lymphoma, and paragranuloma by paraffin immunohistochemistry. *Am J Surg Pathol*. 1998;22:1184–1191.
- Nyuyen PL, Ferry JA, Harris NL. Progressive transformation of germinal centers and nodular lymphocyte predominance Hodgkin's disease: a comparative immunohistochemical study. *Am J Surg Pathol*. 1999;23:27–33.
- Karayalcin G, Behm F, Geiser P, et al. Lymphocyte-predominant Hodgkin's disease: clinicopathologic features and results of treatment. The Pediatric Oncology Group experience. *Med Pediatr Oncol*. 1997;29:519–525.
- Skinninger BF, Mak TW. The role of cytokines in classical Hodgkin lymphoma. *Blood*. 2002;99:4283–4297.
- Caporaso NE, Goldin LR, Anderson WF, et al. Current insight on trends, causes, and mechanisms of Hodgkin's lymphoma. *Cancer J*. 2009;15:117–123.
- Au WY, Gascoyne RD, Gallagher RE, et al. Hodgkin's lymphoma in Chinese migrants to British Columbia: a 25-year survey. *Ann Oncol*. 2004;15:626–630.
- Fromm JR, Thomas A, Wood BL. Flow cytometry can diagnose classical Hodgkin lymphoma in lymph nodes with high sensitivity and specificity. *Am J Clin Pathol*. 2009;131:322–332.
- Stein H, Hummel M. Cellular origin and clonality of classic Hodgkin's lymphoma: immunophenotypic and molecular studies. *Semin Hematol*. 1999;36:233–241.
- Ohno T, Stribley JA, Wu G, et al. Clonality in nodular lymphocyte-predominant Hodgkin's disease. *N Engl J Med*. 1997;337:459–465.
- Marafioti T, Hummel M, Foss HD, et al. Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood*. 2000;95:1443–1450.

41. Stein H, Marafioti T, Foss HD, et al. Down-regulation of BOB.1/OBF.1 and Oct2 in classical Hodgkin disease but not in lymphocyte predominant Hodgkin disease correlates with immunoglobulin transcription. *Blood*. 2001;97:496–501.
42. Kueppers R. Molecular biology of Hodgkin's lymphoma. *Adv Cancer Res*. 2002;84:277–312.
43. Re D, Thomas RK, Behringer K, et al. From Hodgkin disease to Hodgkin lymphoma: biologic insights and therapeutic potential. *Blood*. 2005;105:4553–4560.
44. Re D, Kuppers R, Diehl V. Molecular pathogenesis of Hodgkin's lymphoma. *J Clin Oncol*. 2005;23:6379–6386.
45. Bargou RC, Leng C, Krappmann D, et al. High level nuclear NF-kappa B and Oct-2 is a common feature of cultured Hodgkin/Reed-Sternberg cells. *Blood*. 1996;87:4340–4347.
46. Messineo C, Jamerson MH, Hunter E, et al. Gene expression by single Reed-Sternberg cells: pathways of apoptosis and activation. *Blood*. 1998;91:2443–2451.
47. Kanzier H, Küppers R, Helmes S, et al. Hodgkin and Reed-Sternberg-like cells in B-cell chronic lymphocytic leukemia represent the outgrowth of single germinal-center B-cell-derived clones; potential precursors of Hodgkin and Reed-Sternberg cells in Hodgkin's disease. *Blood*. 2000;95:1023–1031.
48. Yung L, Linch D. Hodgkin's lymphoma. *Lancet*. 2003;361:943–951.
49. Braeuninger A, Hansmann ML, Strickler JG, et al. Identification of common germinal-center B-cell precursors in two patients with both Hodgkin's disease and non-Hodgkin's lymphoma. *N Engl J Med*. 1999;340:1239–1247.
50. Marafioti T, Hummel M, Angnostopoulos I, et al. Classical Hodgkin disease and follicular lymphoma originating from the same germinal center B cells. *J Clin Oncol*. 1999;17:3804–3809.
51. Manis JP. Precursors of Hodgkin's disease and B-cell lymphoma. *N Engl J Med*. 1999;340:1280–1281.
52. Küppers R, Klein U, Schwering I, et al. Identification of Hodgkin and Reed-Sternberg cell-specific genes by gene expression profiling. *J Clin Invest*. 2003;111:529–537.
53. Joos S, Kupper M, Ohl S, et al. Genomic imbalances including amplification of the tyrosine kinase gene JAK2 in CD30+ Hodgkin cells. *Cancer Res*. 2000;60:549–552.
54. Diehl V, Franklin J, Hansmann ML, et al. Clinical presentation, course, and prognostic factors in lymphocyte-predominant Hodgkin's disease and lymphocyte-rich classical Hodgkin's disease: report from the European Task Force on Lymphoma Project on Lymphocyte-predominant Hodgkin's disease. *J Clin Oncol*. 1999;12:776–783.
55. Jackson C, Sirohi B, Cunningham D, et al. Lymphocyte-predominant Hodgkin lymphoma—clinical features and treatment outcomes from a 30-year experience. *Ann Oncol*. 2010;21:2061–2068.
56. Levine AM. Hodgkin's disease in the setting of human immunodeficiency virus infection. *J Natl Cancer Inst Monogr*. 1998;23:37–42.
57. Carbone PP, Kaplan HS, Musshoff K, et al. Report of the committee on Hodgkin's disease staging classification. *Cancer Res*. 1971;31:1860–1861.

CASE 48

Post-transplant Lymphoproliferative Disorders

CASE HISTORY

A 65-year-old man presented with a cutaneous skin lesion on the left forearm for several months. He had a history of left lung transplant 10 years ago due to severe bullous emphysema and had had continued immunosuppression with azathioprine and tacrolimus until the recent visit. One year prior to the current admission, the patient had swelling and redness in the left wrist, and a biopsy showed pyogenic granuloma. The lesion became progressive with tenderness and extended to the left forearm in last several months. The patient also had chills, fatigue, weight loss, nausea, and vomiting. Physical examination showed no lymphadenopathy and organomegaly. A skin biopsy of the left forearm was performed (Fig. 6.48.1). After the diagnosis, the dose of immunosuppressive drugs was reduced. However, the patient died 3 months later.

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The skin biopsy showed that the tumor cells were immunoreactive with CD3 (Fig. 6.48.2), CD4 (Fig. 6.48.3), CD30 (Fig. 6.48.4), and CD43, but were not reactive with

CD5, CD8, CD15, CD20, CD79a, and ALK1. Epstein—Barr virus encoded RNA in situ hybridization (EBER) was also negative.

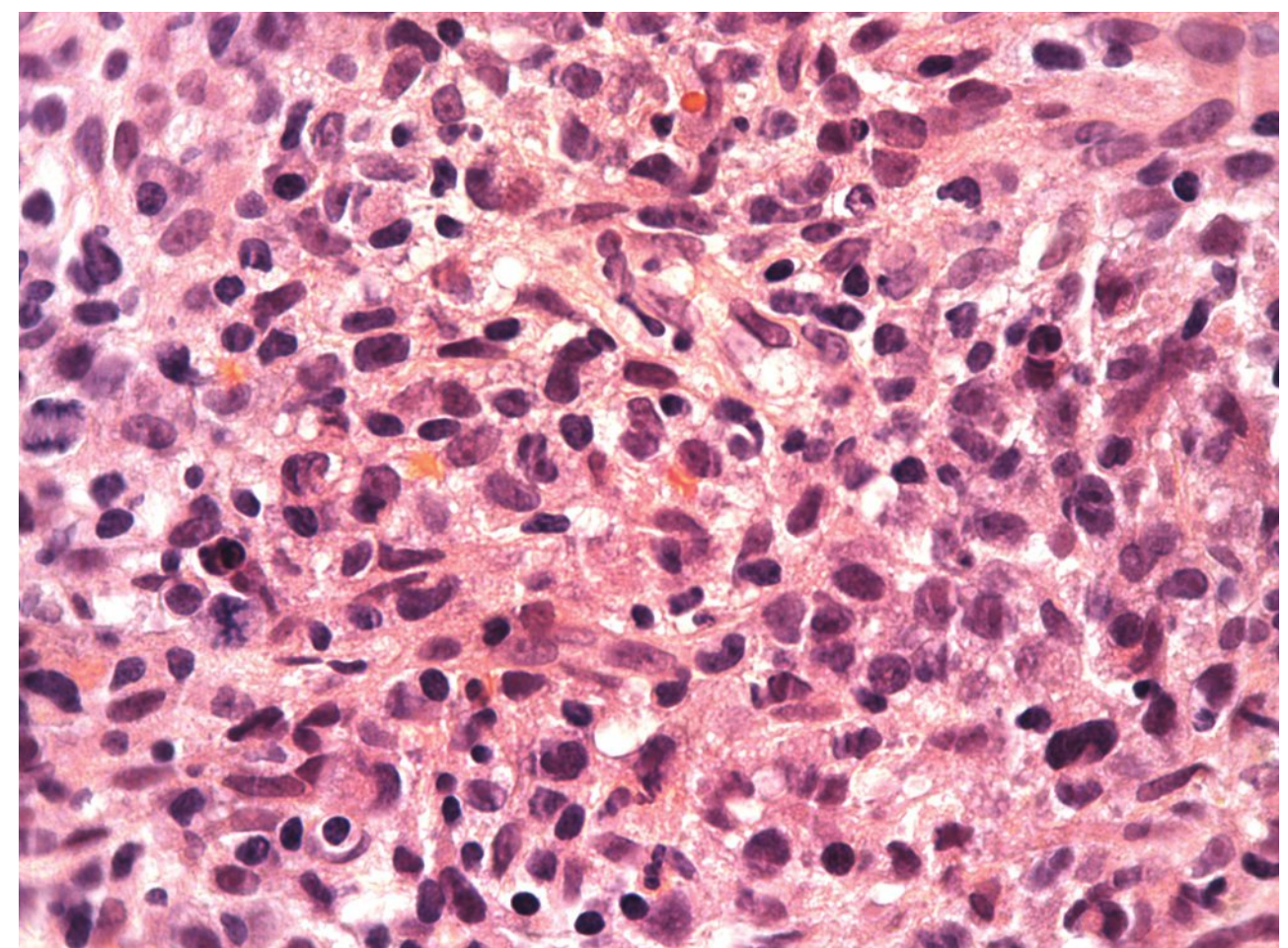


FIGURE 6.48.1 Skin biopsy shows pleomorphic tumor cell infiltration in the dermis. H&E, ×60.

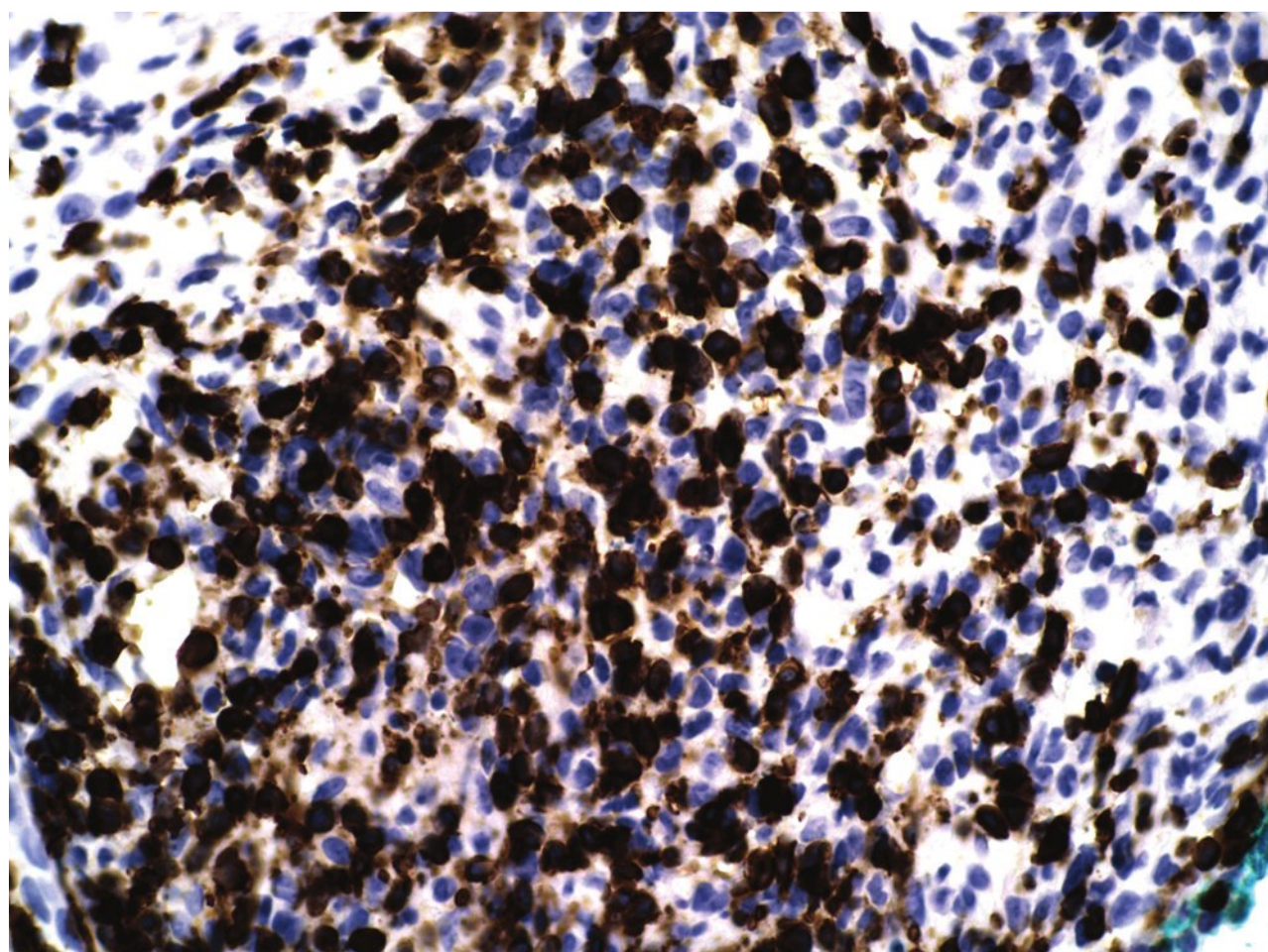


FIGURE 6.48.2 The same biopsy shows positive CD3 staining. Immunoperoxidase, $\times 40$.

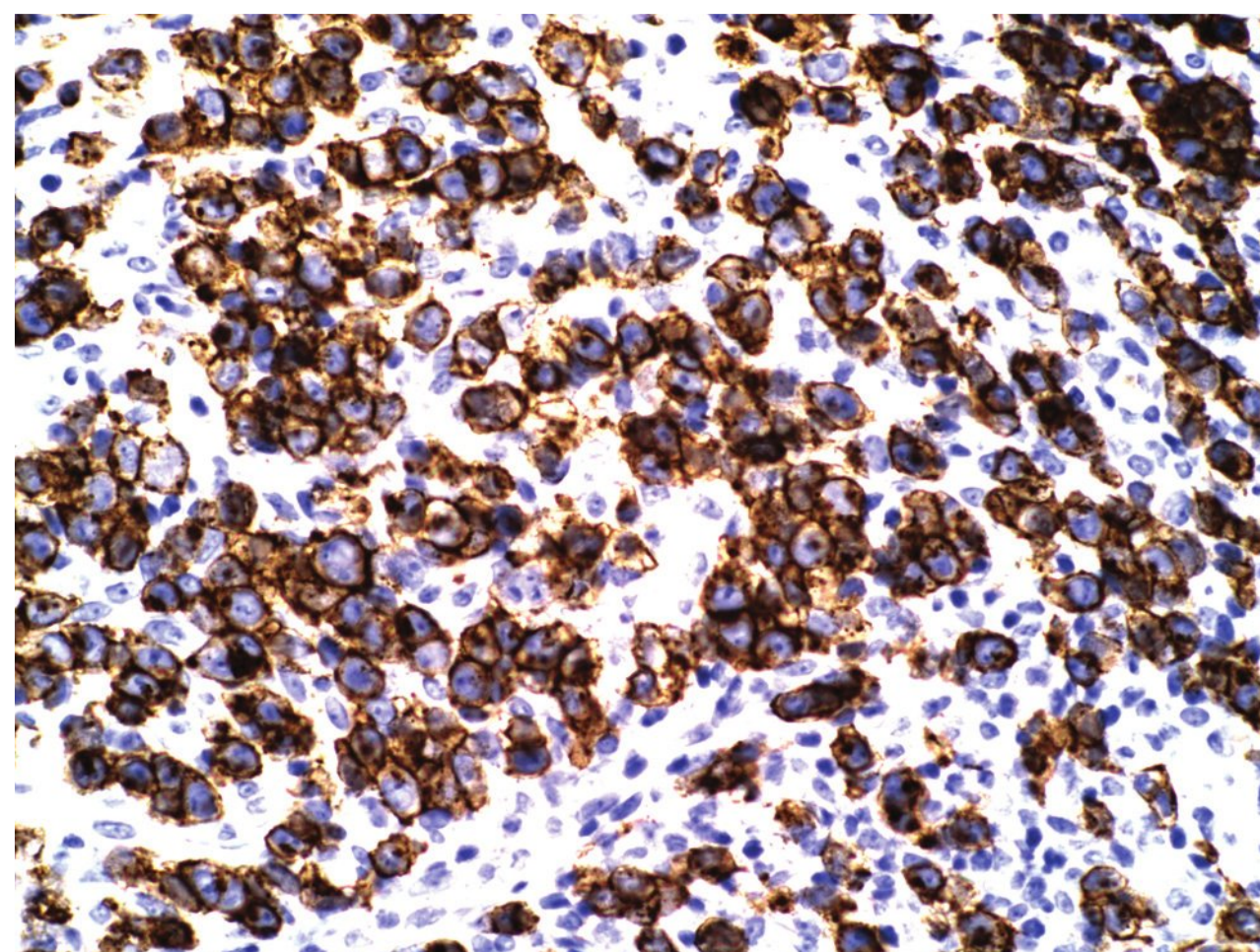


FIGURE 6.48.4 CD30 stain is also positive. Immunoperoxidase, $\times 40$.

DISCUSSION

Post-transplant lymphoproliferative disorders (PTLDs) are lymphoid or plasmacytoid proliferations subsequent to solid organ, bone marrow or stem cell transplantation, as a result of immunosuppression of both tumor surveillance and antiviral activity (1). As will be discussed later, there is a wild spectrum of morphologic changes in this category. The development of PTLD depends on the degree and duration of immunosuppression, the immunosuppressive drugs used, the transplant organ and the original immune status of the patient, including the presence or absence of antibodies against the Epstein—Barr virus (EBV) (1–5).

Most cases of PTLD develop within the first year of transplantation. The early onset PTLD is usually associated with EBV infection (1–4). Bone marrow allograft recipients may develop the disease as early as 6 months after transplant. The incidence of PTLD is decreasing in proportion to the duration of transplantation. The incidence of

PTLD in peripheral stem cell and bone marrow allograft is about 1%; kidney and liver transplants range from 1% to 3%; cardiac transplants, 1% to 6%; combined heart-lung transplants, 4% to 6%; lung transplants, 4% to 10%; and small intestinal transplants, up to 20%.

EBV infection as transmitted from the graft is of particular importance, as EBV may induce B-cell transformation with resultant uncontrolled lymphoproliferation. Approximately 50% of PTLD cases are derived from germinal center (GC) B cells that lack a functional B-cell receptor because of certain crippling mutations (6). These dysfunctional cells are usually destined to programmed cell death, and it is believed that their survival is the result of EBV rescue. The pathogenic role of EBV partly explains the high incidence of PTLD in pediatric patients, because most of children are EBV-naïve recipients. It is advocated that patients with EBV-negative serology should receive prophylactic antiviral treatment to prevent the development of PTLD (3). EBV-positive cases tend to occur earlier than EBV-negative cases do; the former has a medium interval of 6 to 10 months compared with 4 to 5 years for the latter.

Approximately one third of PTLD cases are EBV-negative. These cases are frequently late-onset PTLD and are often associated with monomorphic type of lymphoproliferation (1–4). The etiology may be due to other viruses, such as human herpes virus 8, or chronic antigenic stimulation from the transplanted organ (4–6). Another possibility is that EBV induces mutation in lymphocytes, which then proliferate spontaneously without the presence of EBV (4).

Most PTLD cases in solid organ recipients are of host origin. Less than 10% of cases are of donor origin and are often seen in liver and lung transplant (2,7). On the other hand, PTLD in recipients of bone marrow allograft is commonly of donor origin (2).

Morphology

The World Health Organization (WHO) classification divides PTLD into four categories: early lesions, polymorphic PTLD, monomorphic PTLD and classical Hodgkin

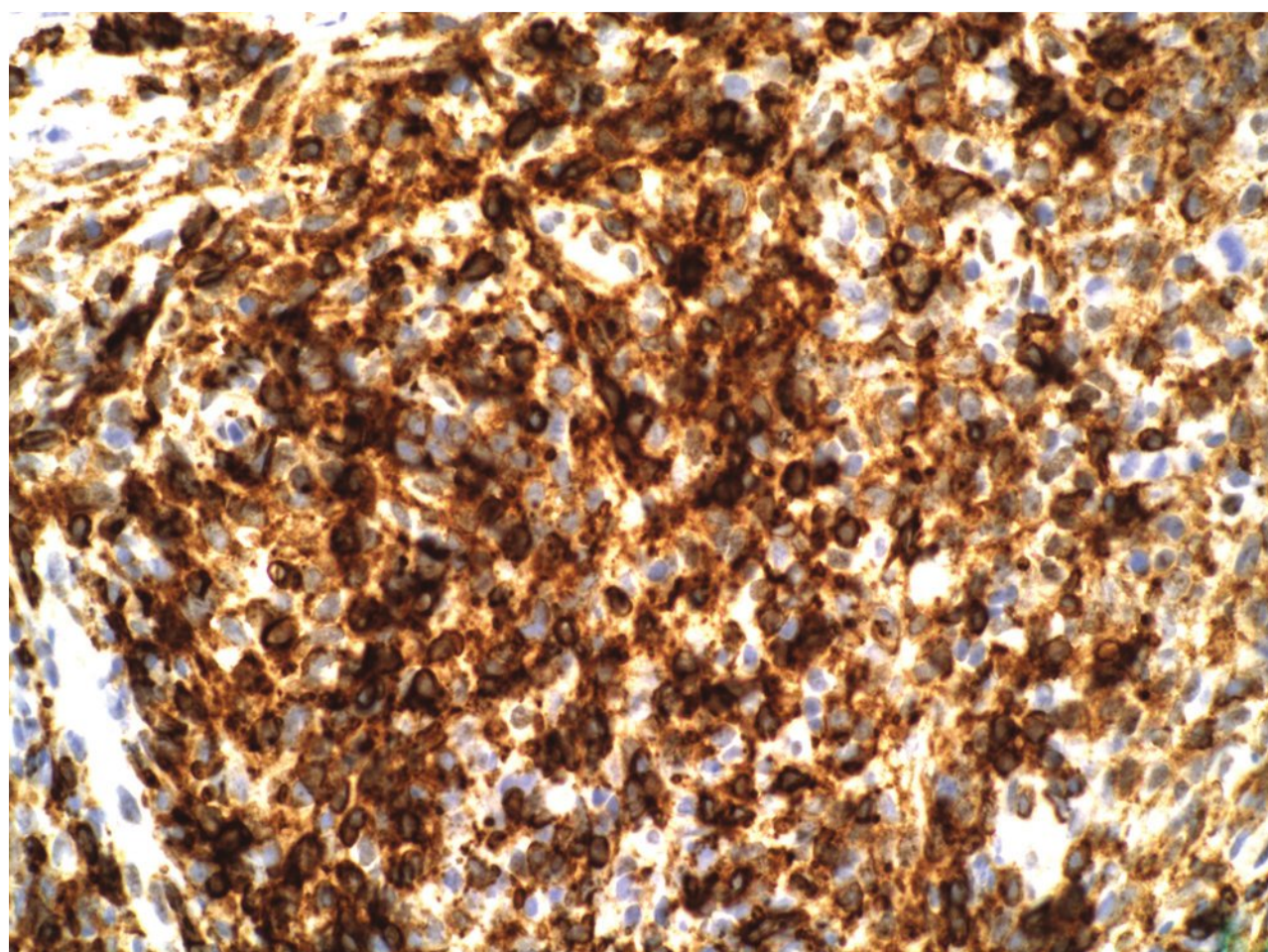


FIGURE 6.48.3 CD4 stain is positive for tumor cells. $\times 40$.

lymphoma type PTLD (2). The major histologic feature in early lesions is nondestructive lymphoplasmacytic proliferation with architectural preservation of the involved tissue. However, most cases show mass lesions. The most common presentations are plasmacytic hyperplasia and infectious mononucleosis-like PTLD. Plasmacytic hyperplasia shows predominantly plasma cells intermixed with small lymphocytes and a few bland-looking immunoblasts. Infectious mononucleosis-like PTLD is characterized by paracortical expansion with numerous immunoblasts in a background of T lymphocytes and plasma cells.

Polymorphic PTLD is defined by the WHO as “morphologically polymorphic lesions composed of immunoblasts, plasma cells and small and intermediate sized lymphoid cells that efface the architecture of lymph nodes or form destructive extranodal masses” (2,8). These lesions do not fulfill the criteria for any lymphomas that are recognized in immunocompetent hosts. The distinction between polymorphic PTLD and early PTLD lesions is the effacement of the architecture of the involved tissue. In contrast to monomorphic PTLD, the cellular population is pleomorphic, consisting of lymphocytes in a full range of maturation, from immunoblasts to small and large lymphocytes to plasma cells. Focal necrosis, Reed-Sternberg-like cells, and a high mitotic rate may be present. Some of these cases were previously described as Hodgkin-like PTLD, but in the light of current studies, most, if not all, of these cases are B-cell PTLD (9). In some cases, monomorphic features can be seen in selected areas. Sometimes, the demarcation between polymorphic and monomorphic PTLDs is blurred.

Monomorphic PTLD is morphologically identical to de novo B-cell or natural killer (NK)/T-cell lymphomas (2,8). As the name implies, most cases show a monotonous proliferation of transformed lymphoid cells or plasma cells. However, in certain cases, pleomorphism can be demonstrated in some areas. Therefore, polymorphic and monomorphic PTLD are considered a spectrum of the same disease rather than two distinct entities. Most cases of monomorphic PTLD are of B-cell lineage, with the majority being diffuse large B-cell lymphoma and less frequently, Burkitt lymphoma or a plasma cell neoplasm (plasma cell myeloma or extramedullary plasmacytoma). Bizarre nuclei, multinucleated giant cells, and Reed-Sternberg-like cells can be seen in these B-cell neoplasms.

T/NK lymphomas account for 4% to 14% of all PTLD cases (2). It covers a wide spectrum of neoplasms, including peripheral T-cell lymphoma, not otherwise specified, hepatosplenic T-cell lymphoma, mycosis fungoides/Sézary syndrome, anaplastic large-cell lymphoma, and NK/T-cell leukemia/lymphoma. The first two entities are most commonly seen. The morphology of these tumors does not differ from that seen in immunocompetent hosts.

Classical Hodgkin lymphoma is the least common form of PTLD (2,8). It is almost always EBV-positive. Since Reed-Sternberg-like cells can be seen in polymorphic and monomorphic PTLDs, a comprehensive study with immunophenotyping and molecular genetics should be conducted before a diagnosis of Hodgkin lymphoma is made.

The major differential diagnoses are allograft rejection and primary lymphoid neoplasms. The identification

of EBV in the tumor cells is helpful to distinguish primary B-cell lymphoma from monomorphic PTLD and to distinguish polymorphic PTLD from rejection of allograft. Heavy-chain gene analysis is useful in establishing the diagnosis of PTLD when a large population of polyclonal B cells is identified by immunophenotyping.

Immunophenotyping

In the early lesions, immunophenotyping may demonstrate a mixed population of polyclonal B cells, plasma cells, and T cells without any phenotypic aberration. The polymorphic PTLD cases may show a polyclonal or monoclonal B-cell population admixed with a heterogeneous T-cell population. The monoclonal B-cell population may be focal or may be multiple. If the monoclonal B-cell population is predominant, monomorphic PTLD should be excluded.

The monomorphic PTLD always shows a monoclonal B-cell or plasma cell population (2,8). As most cases are diffuse large B-cell lymphoma or Burkitt lymphoma, they can be further divided into two subgroups, the GC type or the non-GC type on the basis of a combination of CD10, BCL-6, multiple myeloma oncogene protein 1 (IRF4/MUM1), and CD138 immunophenotype (2,7,10). Cases with either CD10 or BCL-6 expression and negative IRF4/MUM1 and CD138 are considered GC type. On the other hand, cases with negative CD10 and BCL-6 but positive IRF4/MUM1 and/or CD138 are considered late GC/post GC type (non-GC type). Most monomorphic PTLD are of non-GC type. The majority of EBV negative cases are of GC type, while EBV-positive cases are of late GC/post GC type. PTLD cases of donor origin are also of post GC type (7).

Most cases of B-cell lymphoma also express CD30 with or without anaplastic morphology. As mentioned before, Reed-Sternberg-like cells can be seen in all forms of PTLDs; therefore, it is important to perform immunohistochemical stains for CD15, CD30, and CD45 to distinguish Hodgkin from non-Hodgkin lymphomas. These Reed-Sternberg-like cells are usually negative for CD15 and positive for CD45

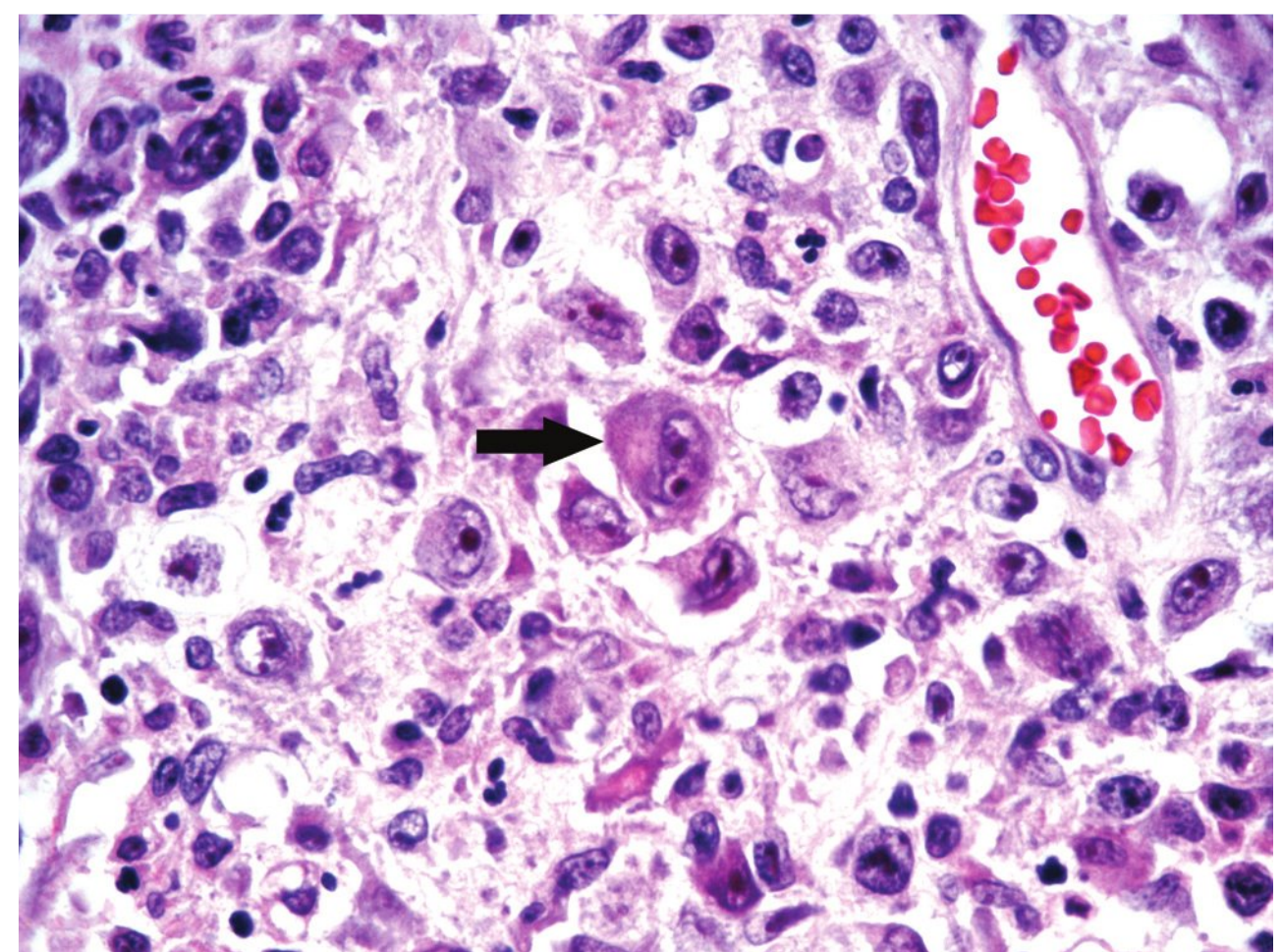


FIGURE 6.48.5 Brain biopsy in a case of PTLD diffuse large B-cell lymphoma shows a Reed-Sternberg-like cell (arrow) in the center of this field. H&E, $\times 60$.

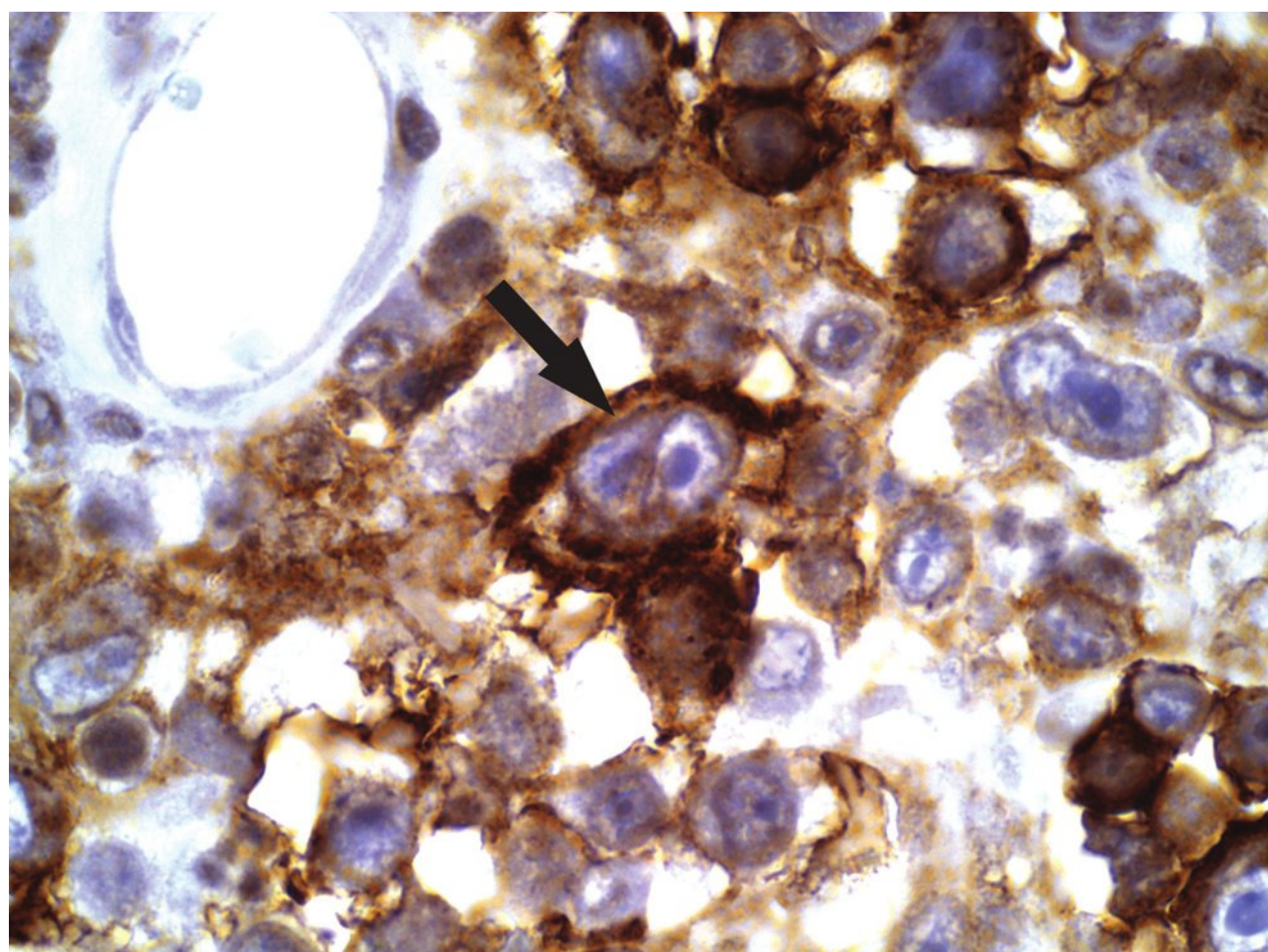


FIGURE 6.48.6 CD30 stain is positive for the Reed-Sternberg-like cell (arrow). Immunoperoxidase, $\times 100$.

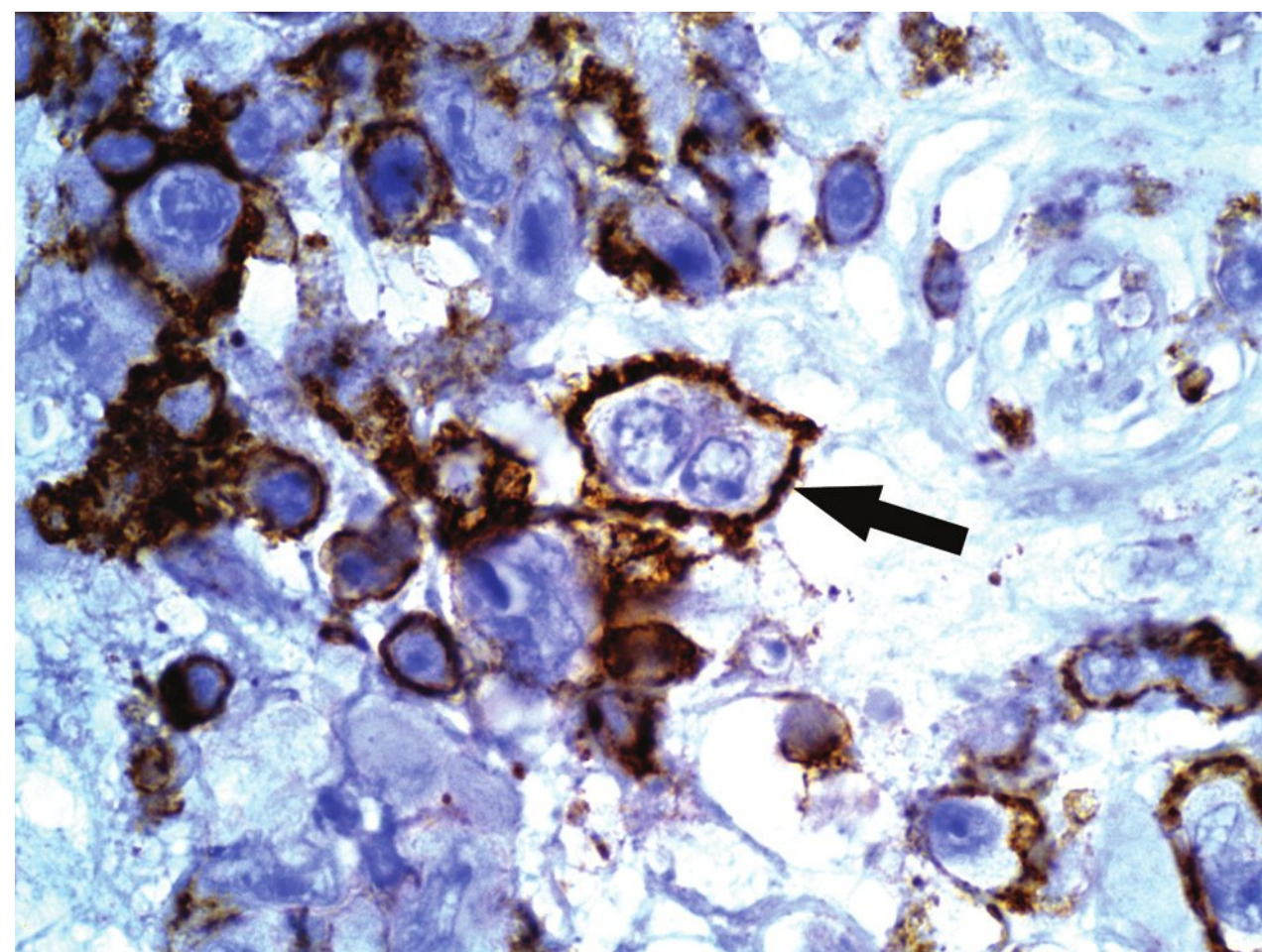


FIGURE 6.48.8 CD45 stain is positive for the Reed-Sternberg-like cells (arrow). Immunoperoxidase, $\times 100$.

and B-cell markers (9,11) (Figs. 6.48.5–6.48.9). One study has also found that these cells are invariably positive for EBV-LMP-1 protein and vimentin (11). The immunophenotype of T/NK-PTLDs is the same as that of their counterparts in immunocompetent hosts.

Identification of EBV is important for the diagnosis of PTLD, as positive EBV stains may substantiate the diagnosis (6,12). EBER is the most sensitive test for EBV detection and should be used routinely (Fig. 6.48.10) (8). In the early lesions, infectious mononucleosis-like cases are typically EBV-positive, but EBV is positive in most but not all cases of plasmacytic hyperplasia (2). EBV identification in polymorphic PTLD can help to distinguish it from allograft rejection (8). In monomorphic PTLD, EBV is positive in most cases of B cell and plasmacytic neoplasms, but only one third of T/NK-PTLD (2). Hodgkin lymphoma is almost always EBV-positive.

Molecular Genetics

Immunoglobulin heavy-chain gene rearrangement usually shows no clonal population in the early lesions (2). However, some cases of infectious mononucleosis-like lesion may demonstrate small monoclonal or oligoclonal populations. In polymorphic PTLD, clonal immunoglobulin heavy-chain gene rearrangement is present, but is less predominant than in monomorphic PTLD. Seventy-five percent of polymorphic PTLD cases show mutated variable region of the immunoglobulin heavy-chain gene (V_H gene) without ongoing mutations and 25% are unmutated (2). In monomorphic PTLD, immunoglobulin heavy-chain gene rearrangement is clonal in essentially all cases (Fig. 6.48.11), and most cases also show somatic mutation in V_H gene (2,10). In T-cell PTLD, clonal T-cell receptor gene rearrangement may be demonstrated (2). Clonality of the tumor cells can also be demonstrated by the study of the

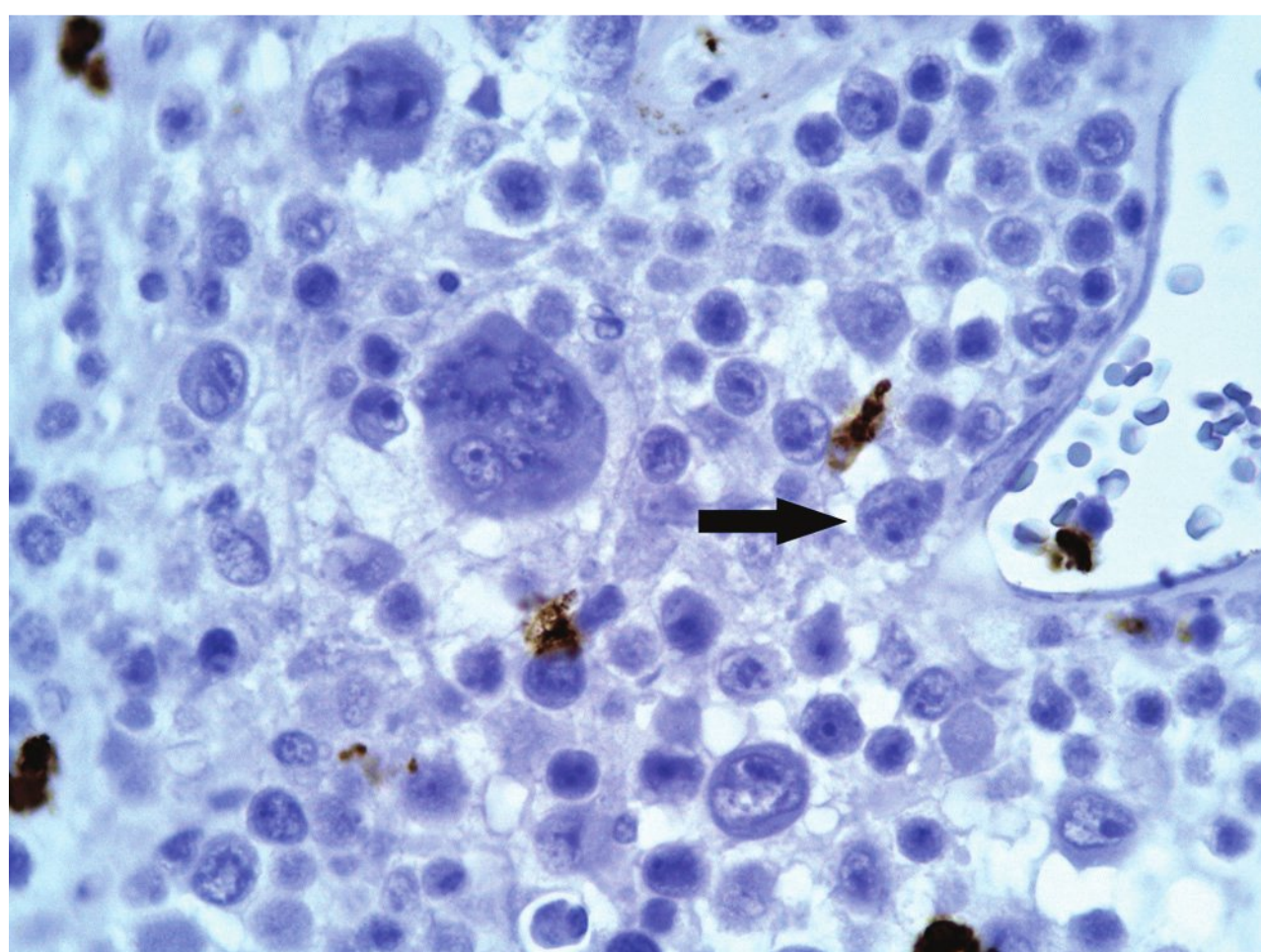


FIGURE 6.48.7 CD15 stain is negative for the Reed-Sternberg-like cell (arrow) and several giant tumor cells. Immunoperoxidase, $\times 60$.

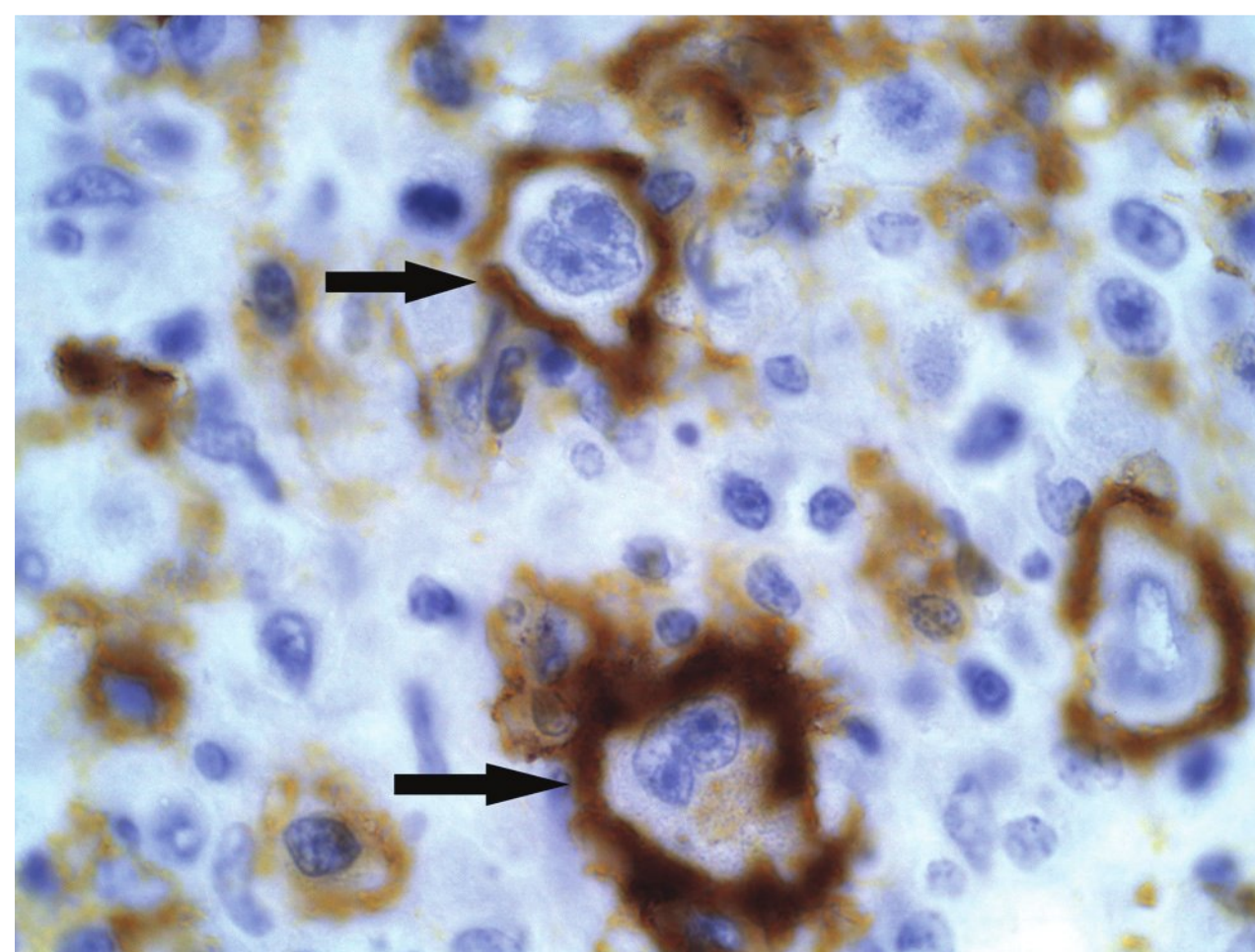


FIGURE 6.48.9 CD20 stain for two Reed-Sternberg-like cells (arrows). Immunoperoxidase, $\times 100$.

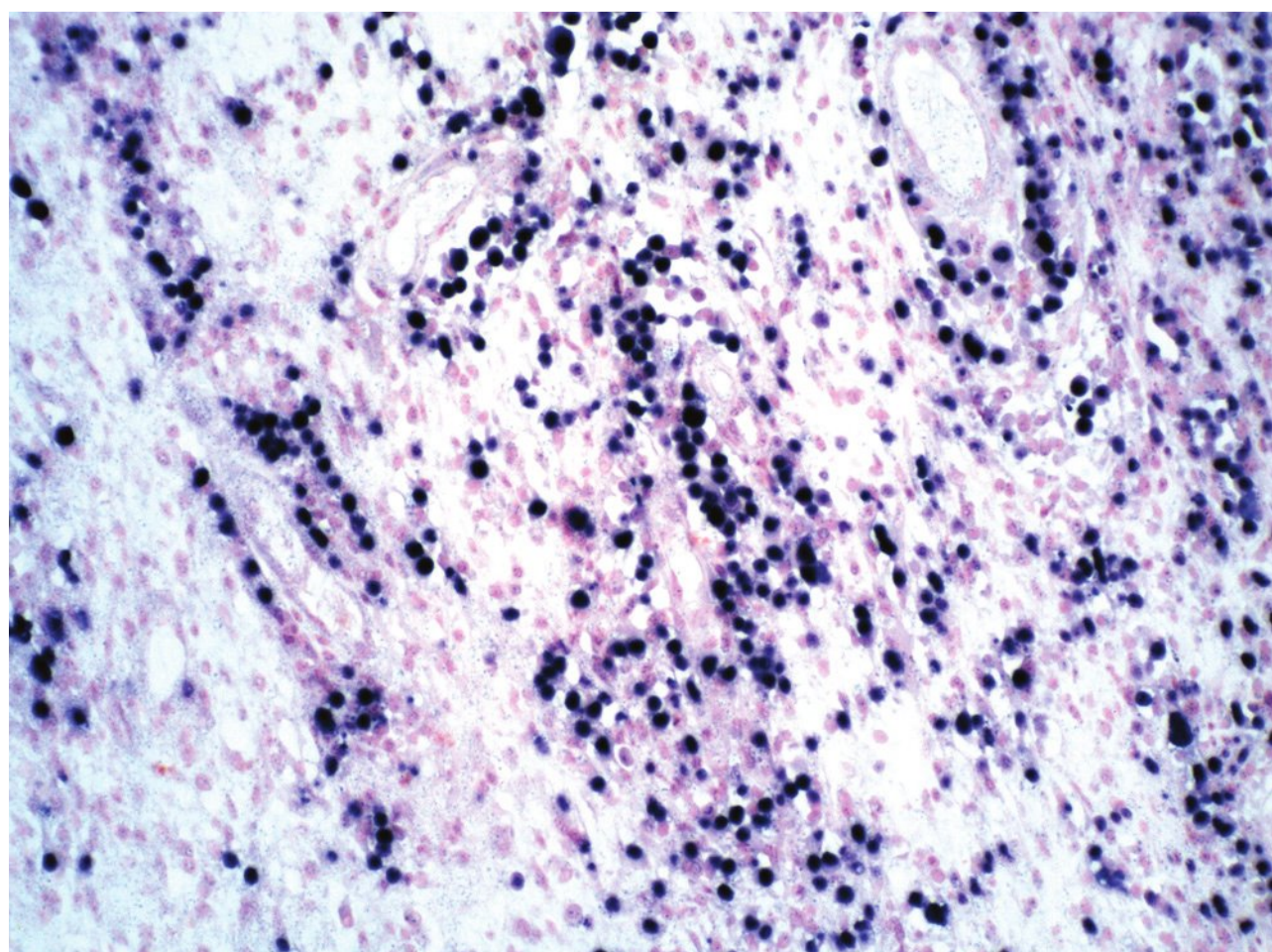


FIGURE 6.48.10 EBER stain is positive for tumor cells, $\times 20$.

episomal form of EBV genome (13). Chromosomal aberrations are most frequently demonstrated in monomorphic PTLD and less frequently in polymorphic PTLD (2). Abnormal karyotypes are seldom seen in early lesions.

The current case is a cutaneous lymphoma with an immunophenotype characteristic for anaplastic large-cell

lymphoma (CD3+ CD4+, CD8–, CD30+, CD15–, CD20–, ALK–). As there was no lymph node involvement with the negative ALK reaction, this is considered to be primary cutaneous anaplastic large-cell lymphoma. Lymphomatoid papulomatosis can be excluded by the aggressive clinical course. The history of lung transplant and the use of high dose immunosuppressive drugs (azathioprine and tacrolimus) for 10 years led to the diagnosis of PTLD. The negative EBER stain does not rule out PTLD as late-onset PTLD is usually EBV negative, particularly for T-cell lymphomas. The reduction of immunosuppressive drug is more effective in early lesions and polymorphic PTLD and less effective in monomorphic PTLD cases, such as the current patient.

PTLD T-cell lymphoma accounts for 0.5% to 20% of all PTLD cases from various reports (14). Post-transplant cutaneous T-cell lymphoma is extremely rare; only 14 cases have been report until 2005. The most common forms of this entity are mycosis fungoides and primary cutaneous CD30+ large T-cell lymphomas. PTLD cutaneous T-cell lymphomas have worse prognosis than their counterparts in immunocompetent patients and similar to that observed in systemic T-cell lymphomas in transplant recipients. Overall, cutaneous lymphomas develop later after transplantation than extracutaneous lymphomas. The salient features for laboratory diagnosis of PTLD are summarized in Table 6.48.1.

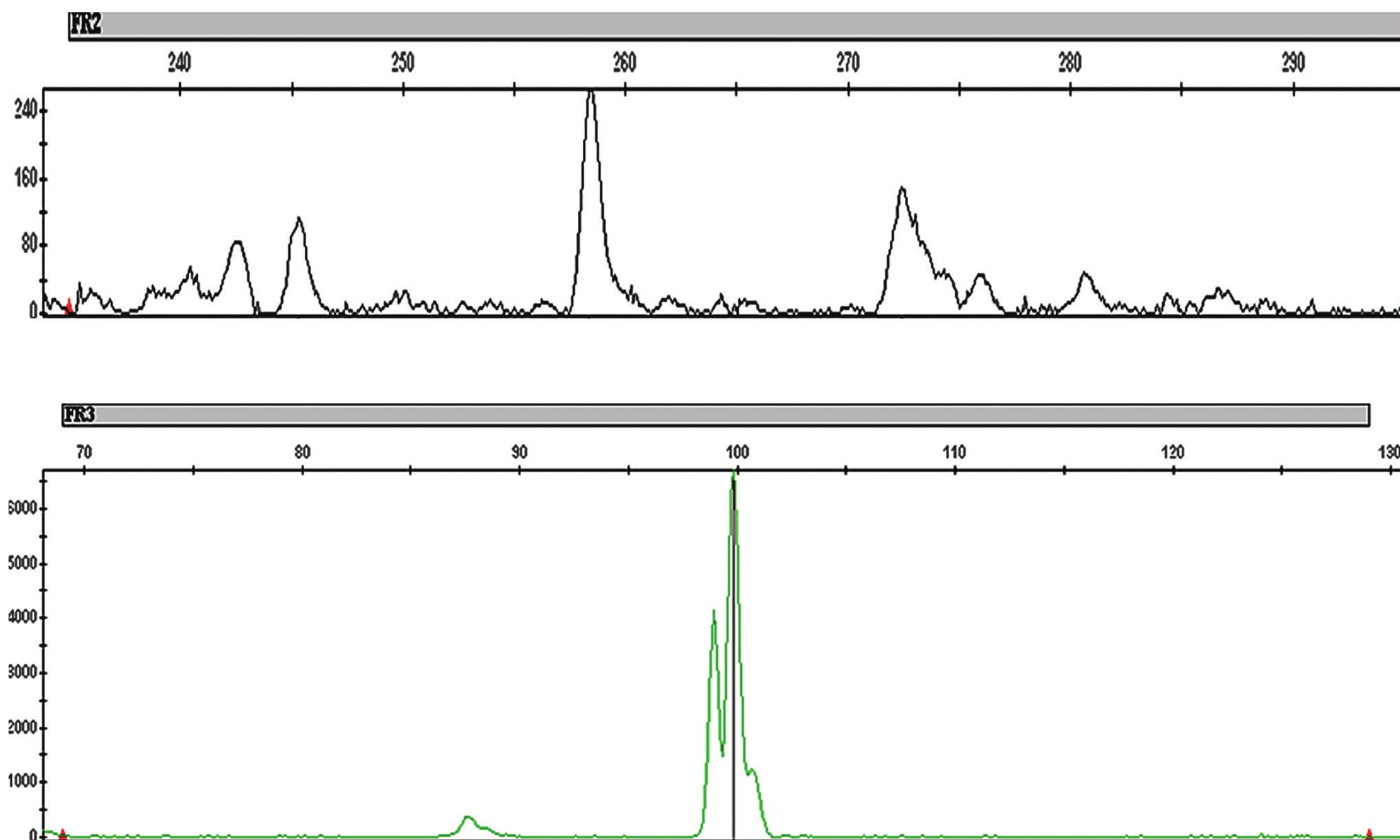


FIGURE 6.48.11 Histogram of polymerase chain reaction with capillary electrophoresis demonstrates a monoclonal spike in frameworks two and three from paraffin sections of brain tissue. (From Sun T. Atlas of Hematologic Neoplasms. Springer, 2009.)

TABLE 6.48.1

Salient Features for Laboratory Diagnosis of PTLDs

1. Histology is the basis for classification of early lesions, polymorphic PTLD, monomorphic PTLD, and Hodgkin lymphoma-type PTLD.
2. Immunophenotyping is used to define cell lineage and clonality. Monoclonal population is usually identified in monomorphic PTLD and in focal areas of polymorphic PTLD, but not in early lesions and Hodgkin lymphoma.
3. EBV is identified in all Hodgkin lymphoma, most cases of infectious mononucleosis-like lesion, B cell and plasmacytic neoplasms, and about one third of T/NK-PTLD. It helps to distinguish allograft rejection and de novo B-cell lymphomas.
4. Immunoglobulin heavy-chain gene rearrangements may help to distinguish some cases of infectious mononucleosis-like lesions and polymorphic PTLD from allograft rejection and nonspecific inflammation.
5. Chromosomal aberrations are most frequently demonstrated in monomorphic PTLD and less frequently in polymorphic PTLD. Abnormal karyotypes are seldom seen in early lesions.
6. EBV genome study may help to establish monoclonality in some cases.

Clinical Manifestations

The clinical features of PTLD correlate with the allograft type, the morphologic entity, and the age of patients. For instance, bone marrow allograft recipients have higher mortality than patients with solid organ transplantation. Pediatric patients fare better than adult patients. Patients with early lesions have a better prognosis than those with polymorphic or monomorphic PTLD. Clinical symptoms are usually nonspecific; patients may have malaise, lethargy, weight loss, and fever. Most cases of PTLD are extranodal, but lymph nodes, gastrointestinal tract, lungs, and liver are relatively more frequently involved (2). The incidence of involvement in the central nervous system varies from rare to 30% in different reports (3,15). Lesions in cutaneous tissue are rare. Depending on the site of involvement, organ-specific symptoms may be demonstrated. Treatments include reduction of immunosuppression,

chemotherapy, and monoclonal antibody (such as rituximab or anti-CD20) therapy (1–4). In EBV-positive patients, antiviral therapy or cellular immunotherapy to restore EBV-specific cytotoxic T-cell immunity can be helpful.

REFERENCES

1. Buell JF, Gross TG, Woodle S. Malignancy after transplantation. *Transplantation*. 2005;80:S254–S264.
2. Swerdlow SH, Webber SA, Chadburn A, et al. Post Transplant lymphoproliferative disorders. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008:343–349.
3. LaCasce AS. Post-transplant lymphoproliferative disorders. *Oncologist*. 2006;11:674–680.
4. Zafar SY, Howell DN, Gockerman JP. Malignancy after solid organ transplantation: an overview. *Oncologist*. 2008;13:769–778.
5. Opetz G, Döhler. Lymphomas after solid organ transplantation: a collaborative transplant study report. *Am J Transplant*. 2003;4:222–230.
6. Rezk SA, Weiss LM. Epstein-Barr virus-associated lymphoproliferative disorders. *Hum Pathol*. 2007;38:1293–1304.
7. Capello D, Rasi S, Oreste P, et al. Molecular characterization of post-transplant lymphoproliferative disorders of donor origin occurring in liver transplant recipients. *J Pathol*. 2009;218(4):478–486.
8. Wallace WAH, Bellamy COC, Rassl DM, et al. Transplant histopathology for the general histopathologist. *Histopathology*. 2003;43:313–322.
9. Pitman SD, Huang Q, Zuppan CW, et al. Hodgkin lymphoma-like posttransplant lymphoproliferative disorder (HL-like PTLD) simulates monomorphic B-cell PTLD both clinically and pathologically. *Am J Surg Pathol*. 2006;30:470–476.
10. Capello D, Cerri M, Muti G, et al. Molecular histogenesis of posttransplantation lymphoproliferative disorders. *Blood*. 2003;102:3775–3785.
11. Chetty R, Biddolph S, Gatter K. An immunohistochemical analysis of Reed-Sternberg-like cells in posttransplantation lymphoproliferative disorders. *Hum Pathol*. 1997;28:493–498.
12. Carbone A, Gloghini A, Dotti G. EBV-associated lymphoproliferative disorders: Classification and treatment. *Oncologist*. 2008;13:577–585.
13. Kaplan MA, Ferry JA, Harris NL, et al. Clonal analysis of posttransplant lymphoproliferative disorders, using both episomal Epstein-Barr virus and immunoglobulin genes as markers. *Am J Clin Pathol*. 1994;101:590–596.
14. Lok C, Viseux V, Denoeux JP, et al. Post-transplant cutaneous T-cell lymphomas. *Oncol Hematol*. 2005;56:137–145.
15. Castellano-Sanchez AA, Li S, Qian J, et al. Primary central nervous system posttransplant lymphoproliferative disorders. *Am J Clin Pathol*. 2004;121:246–253.

CASE 49

Langerhans Cell Neoplasm

CASE HISTORY

A 4-year-old boy presented with a swollen left knee for a few weeks. He also had a low-grade fever. Antibiotic and physical therapy did not provide any relief of the local symptoms. Examination of the left knee joint showed swelling, redness, and tenderness. There was no lymphadenopathy and no hepatosplenomegaly. Peripheral blood examination showed mild leukocytosis, while the red cell parameters were within normal ranges. A bone survey revealed punched-out lytic lesions in the left lower shaft of the femur as well as in the skull. A biopsy of the femur was performed (Figs. 6.49.1–6.49.3).

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The bone biopsy showed that the tumor cells were positive for CD1a (Fig. 6.49.4), S100 (Fig. 6.49.5), CD68 (Fig. 6.49.6), and vimentin (Fig. 6.49.7), but were negative for cytokeratin, CD3, CD20, and EMA.

DISCUSSION

Langerhans cell (LC) was first recognized by Paul Langerhans in 1868, but he misidentified it as a neuronal cell because of the presence of dendritic processes (1). During the 1940s, the concept of LC being a tissue macrophage with an immune

function of antigen presentation was finally established. Later on, abnormal histiocytosis was recognized in three clinical entities: eosinophilic granuloma of bone, Letterer—Siwe disease, and Schüller—Christian disease; and Lichtenstein grouped them together as “histiocytosis X (HX)” (2).

The discovery that the granules found in the abnormal histiocytes in the lung and bone lesions in HX are identical to the Birbeck granules in the normal skin Langerhans cell finally brought the new concept of Langerhans cell histiocytosis (LCH) to replace the old term HX (3). Whether LCH is of benign or malignant nature is still controversial. However, at least a small percentage of cases are clearly malignant morphologically and clinically. Those cases are designated Langerhans cell sarcoma.

According to the Histiocyte Society, Langerhans cell is classified in the dendritic cell family, which also includes interdigitating dendritic cell, dermal dendrocyte, and follicular dendritic cell (4). Another classification divides dendritic cells into CD34+ myeloid lineage, which includes Langerhans dendritic cells and interstitial dendritic cells, and CD34+ lymphoid lineage that is solely represented by plasmacytoid dendritic cells (5). The most recent theory presented in the fourth edition of WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues states that Langerhans cells, interdigitating dendritic cells, and dermal/interstitial dendritic cells are myeloid-derived (6). Plasmacytoid dendritic cells are most likely of myelomonocytic lineage. Follicular dendritic cells are stromal-derived, probably from the fibroblastic reticulum cells.

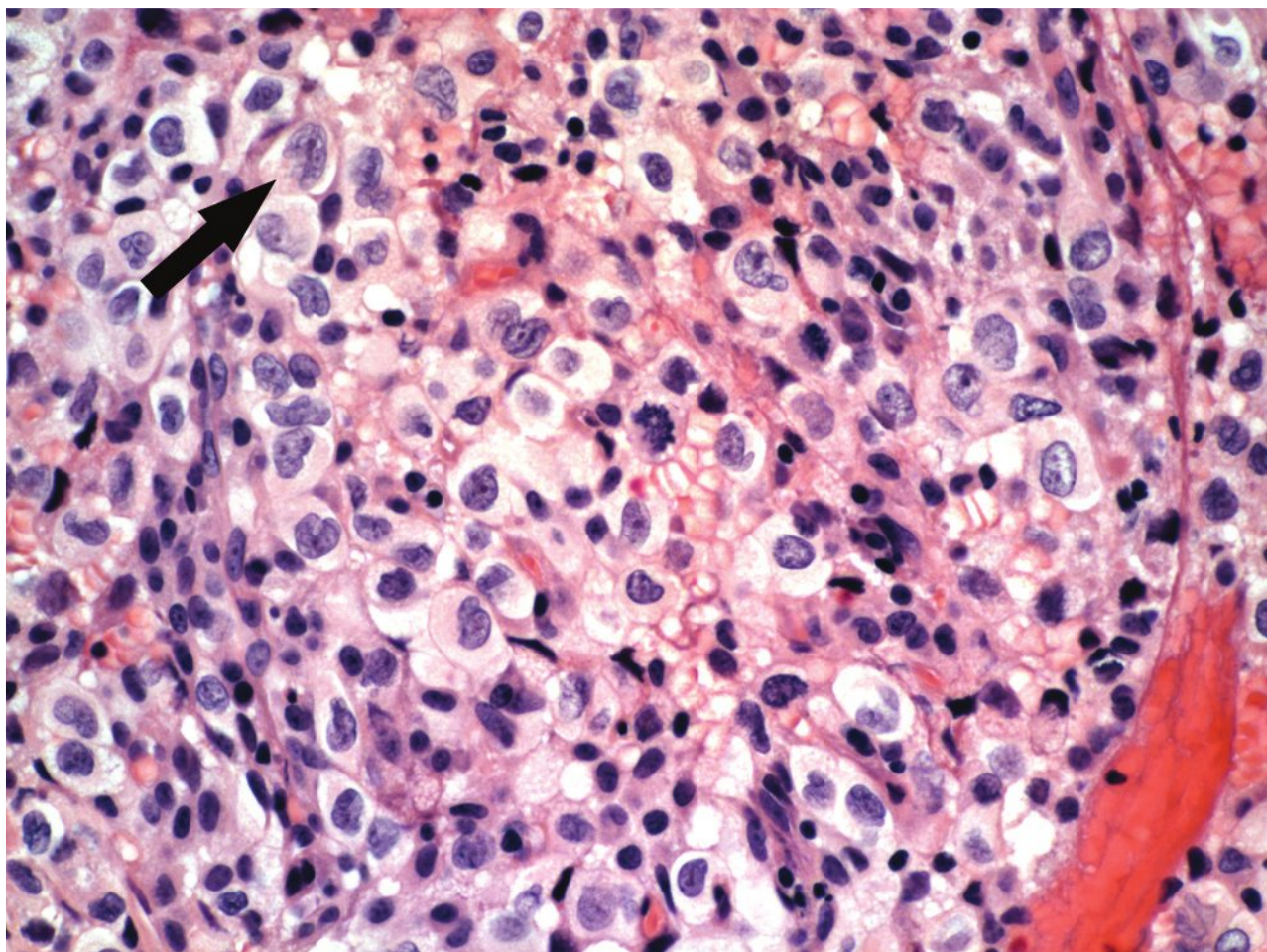


FIGURE 6.49.1 Biopsy of the femur shows extensive Langerhans cell infiltration, intermingled with lymphocytes. Note the variable nuclear configuration of the Langerhans cells with a grooved nucleus pointed by an arrow. The cytoplasm is pale eosinophilic or transparent. H&E, ×40.

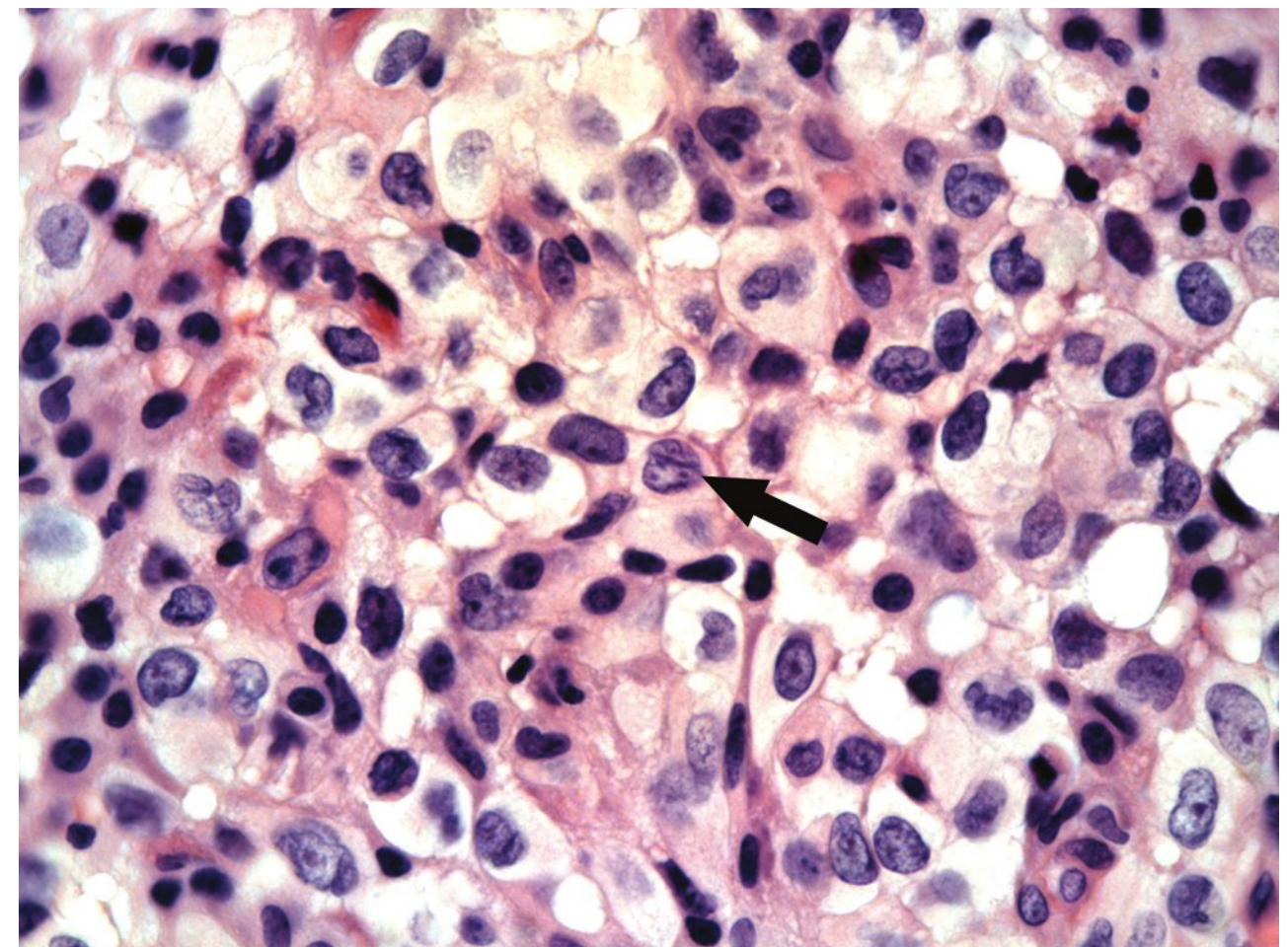


FIGURE 6.49.2 Higher magnification of bone biopsy demonstrates the grooved (arrow), folded, indented or lobulated nuclei of the Langerhans cells. H&E, ×60.

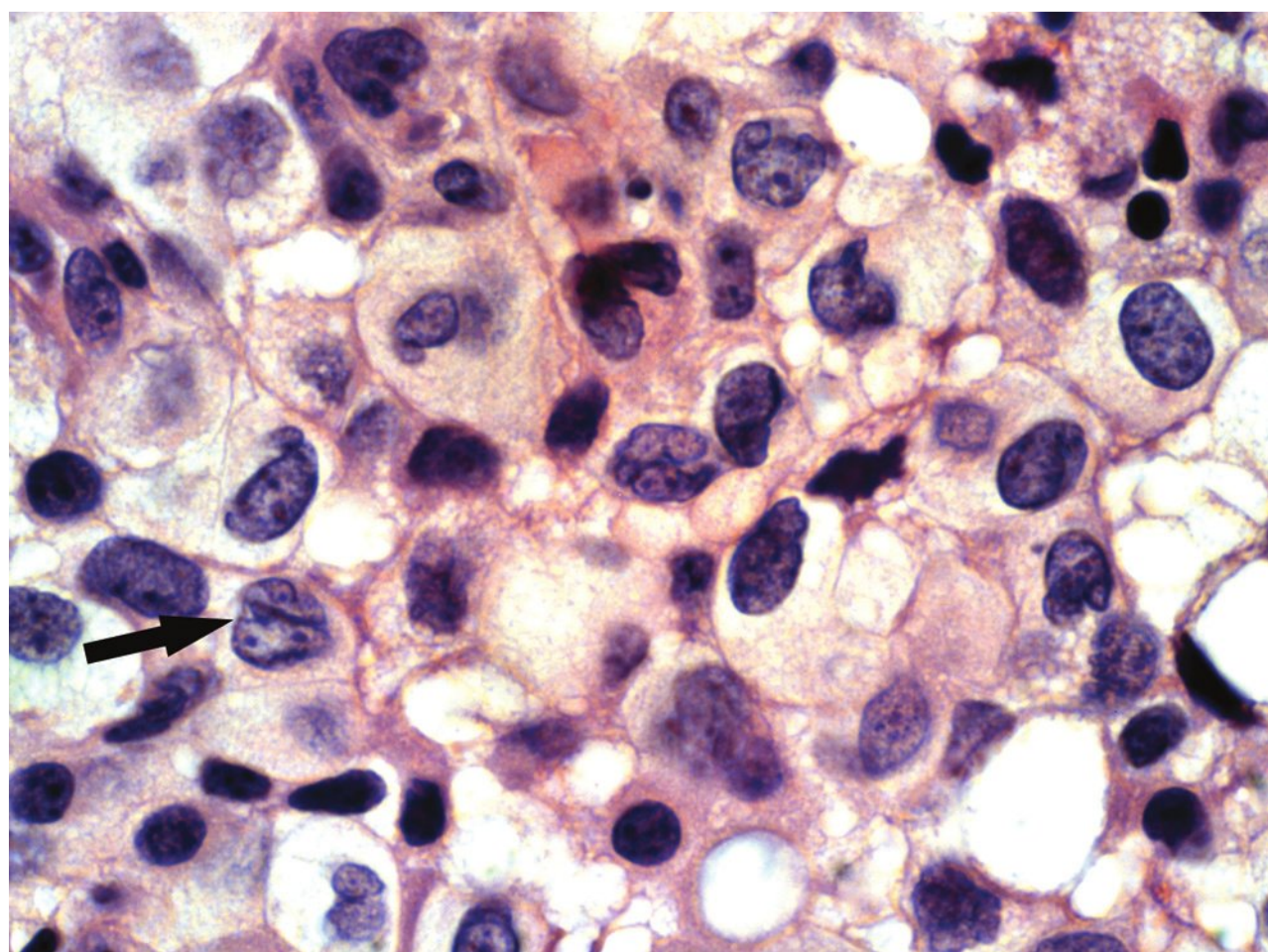


FIGURE 6.49.3 Higher magnification of bone biopsy demonstrates the variable nuclear configuration and clear cytoplasm of the Langerhans cells. A grooved nucleus is indicated by an arrow. H&E, $\times 100$.

Morphology

The diagnosis of LCH mainly depends on the identification of the typical cytology of the Langerhans cells (6–8). The characteristic feature is in the nuclei, which are grooved, folded, indented, or lobulated. The chromatin pattern is fine, the nuclear membrane is thin, and nucleoli are inconspicuous. The tumor cells are oval in shape with moderately abundant eosinophilic cytoplasm and are about 10 to 15 μm .

In early lesions, the predominant population is the Langerhans cells intermixing with eosinophils, neutrophils, lymphocytes, histiocytes, and multinucleated giant cells. Plasma cells are seldom seen. In rare cases, eosinophilic abscesses with central necrosis and Charcot–Leyden crystals may be found. In late lesions, Langerhans cells

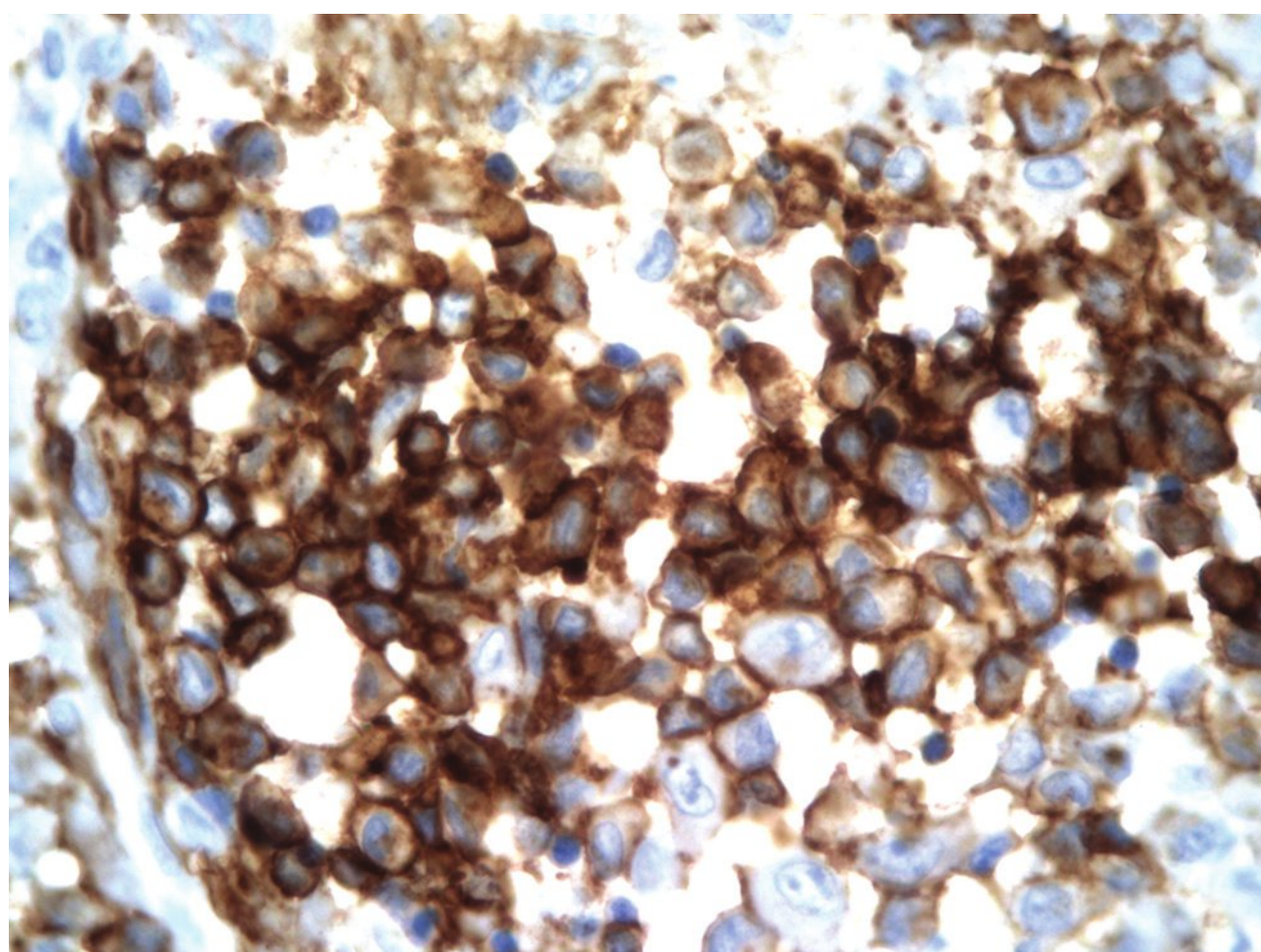


FIGURE 6.49.4 Bone biopsy shows positive CD1a staining on the Langerhans cells. Immunoperoxidase stain, $\times 60$. (From Sun T. Atlas of Hematologic Neoplasms. Springer, 2009.)

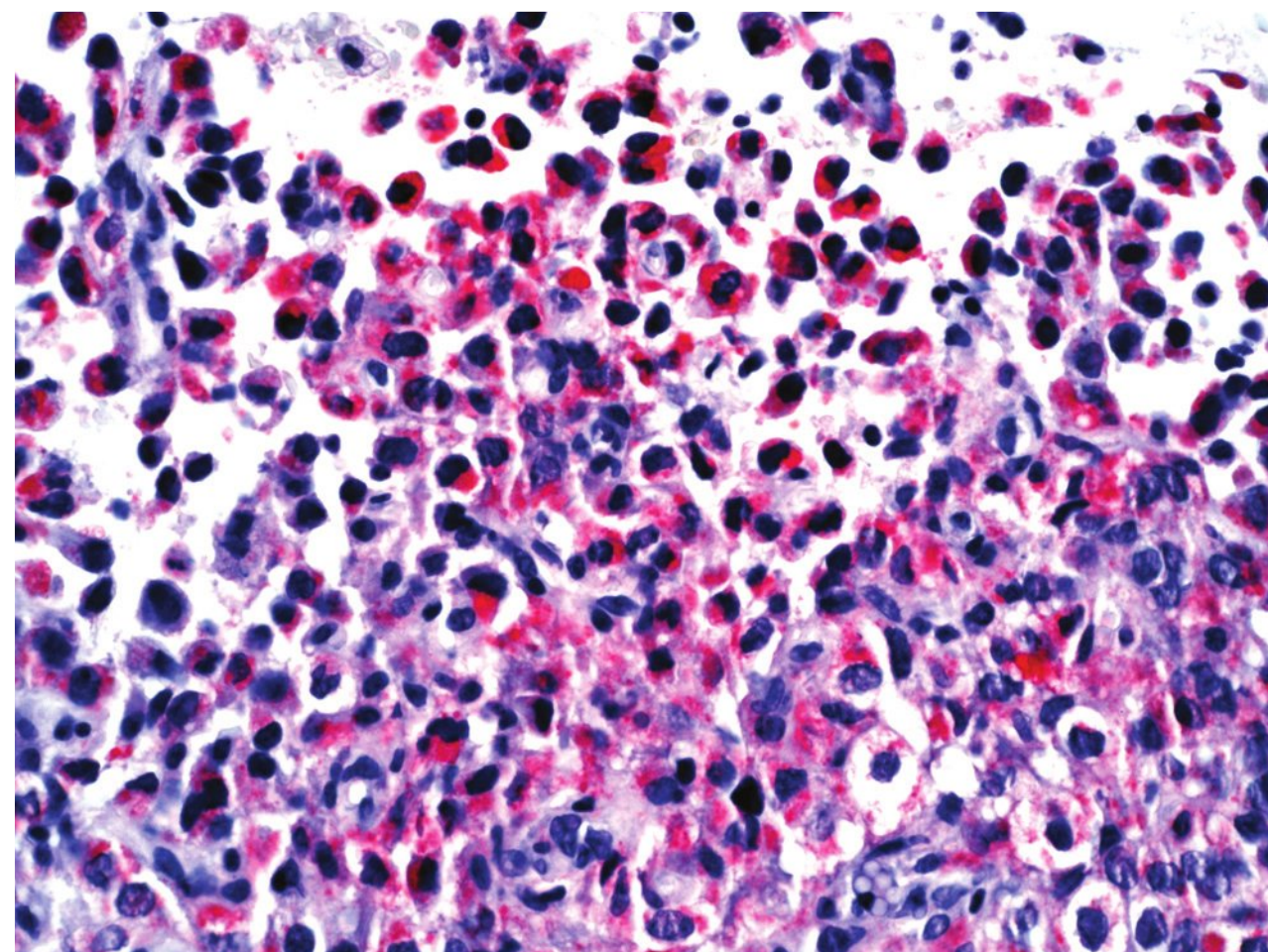


FIGURE 6.49.5 Bone biopsy reveals positive S100 staining on Langerhans cells. Immune-alkaline phosphatase, $\times 40$.

gradually disappear with increases of foamy macrophages and fibrosis.

Mitotic activities are variable in LCH. However, if there is a very high mitotic rate, for example, more than 50 per 10 high power fields, Langerhans cell sarcoma should be considered (6). In those cases, the tumor cells are clearly pleomorphic with prominent nucleoli. In some cases, the tumor cells are no longer recognizable as Langerhans cells. The final diagnosis depends on immunophenotyping and electron microscopic examination to identify the Birbeck granules in the cytoplasm of the tumor cells.

Birbeck granules are racquet-shaped with a double outer sheath and a central striated line, within which there is a zipper-like structure. The entire granule is 200 to 400 nm long and 3.3 nm wide. Langerhans cells lack interdigitating cell processes or cell junctions, which can help to distinguish them from other dendritic cells.

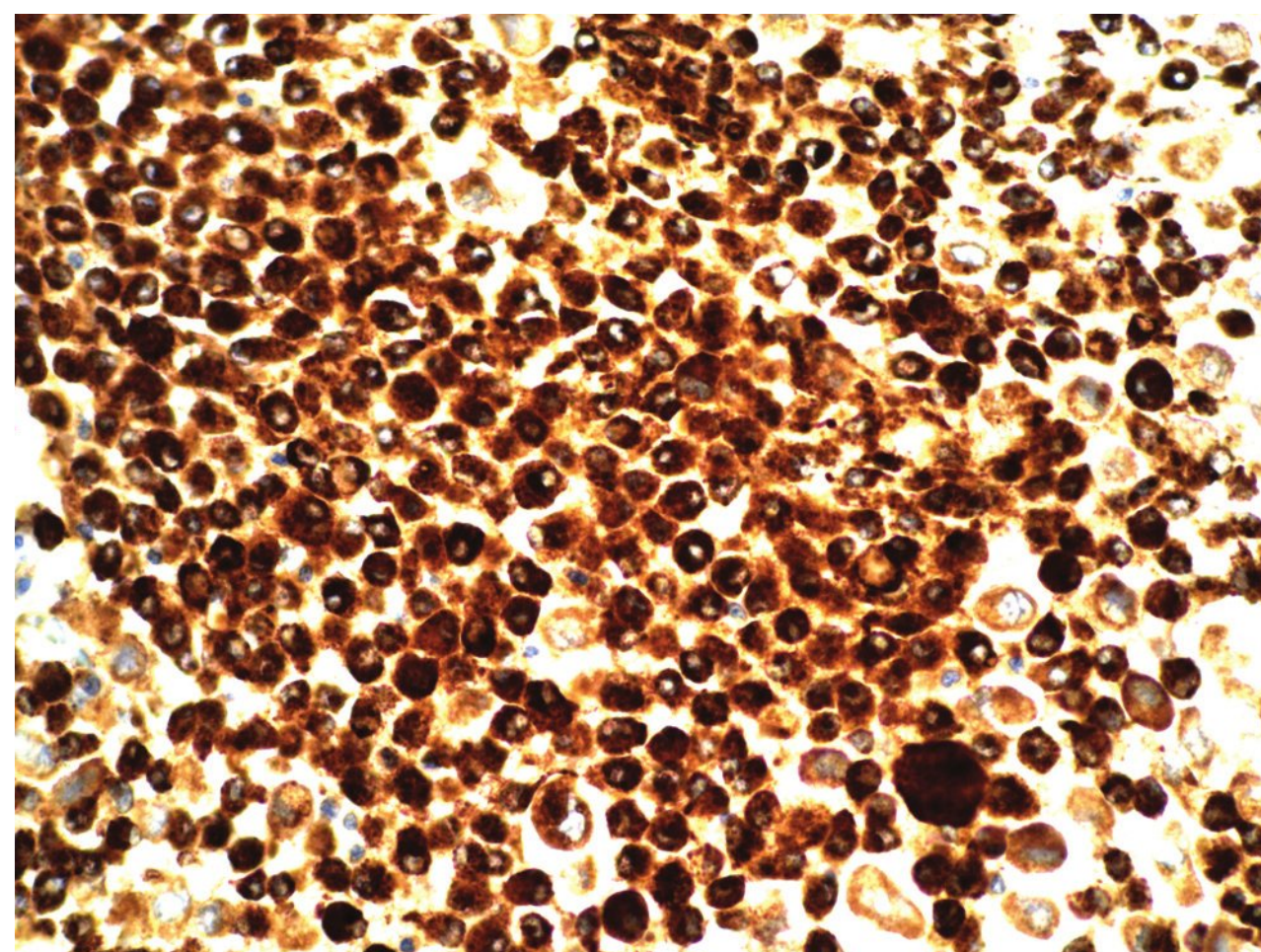


FIGURE 6.49.6 The tumor cells are positive for CD68. Immunoperoxidase, $\times 40$.

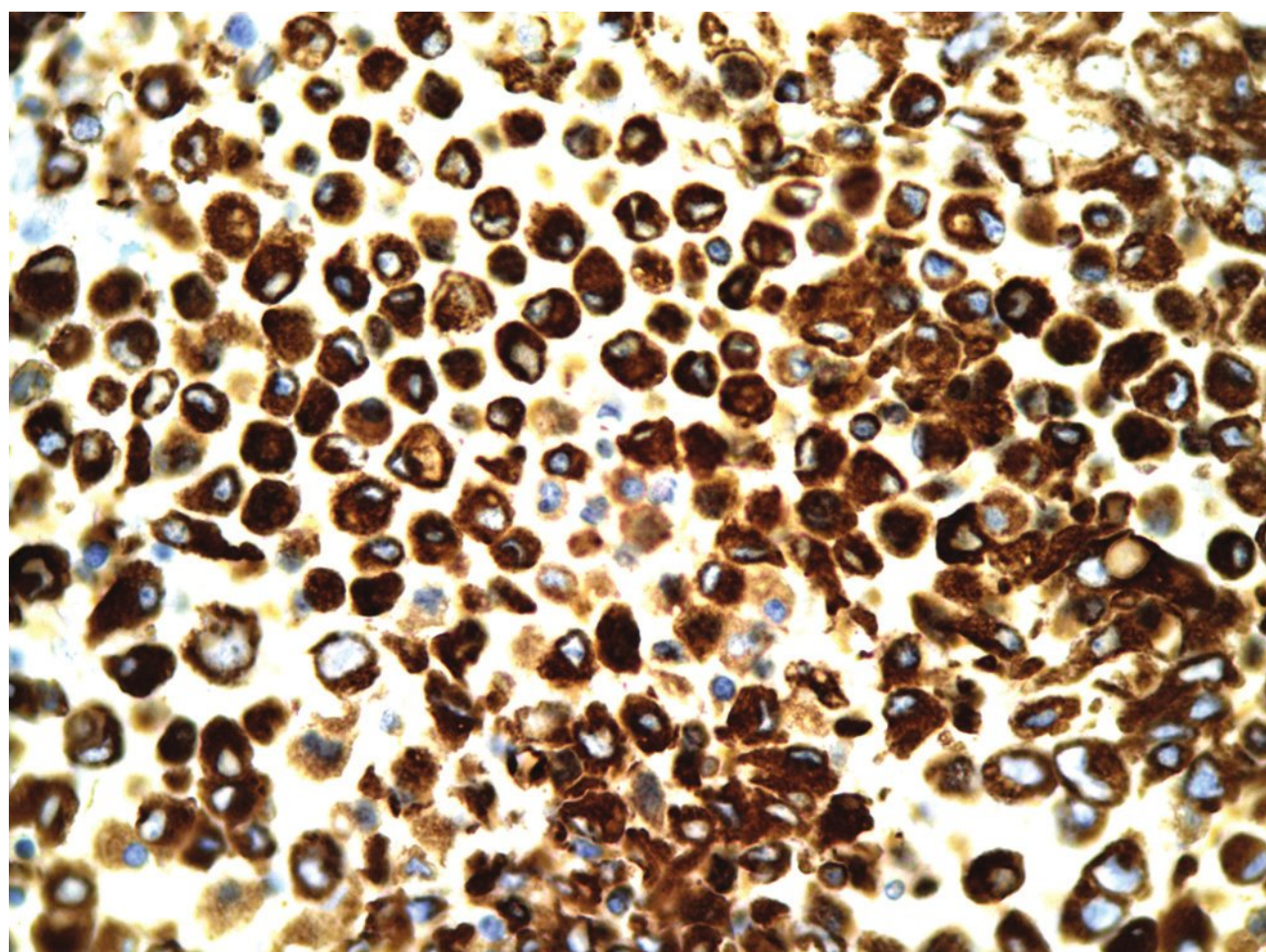


FIGURE 6.49.7 The tumor cells are positive for vimentin. Immunoperoxidase, $\times 60$.

In the lymph nodes, LCH usually involves the sinuses without disruption of the nodal architecture in early cases (6,7). In the later stage, the normal architecture of the lymph node may be partially or totally effaced with necrosis (7). In rare cases, epithelioid granulomas composed of Langerhans cells can be identified in the lymph node (9). In the bone, LCH usually presents as a destructive lesion with predominantly Langerhans cells with the background cells similar to other lesions. Osteoclast-type cells are characteristically seen in bone lesions. The skin lesions usually show periappendageal infiltration. The spleen may show nodular red pulp involvement. The liver lesion is mainly LCH cell infiltration of intrahepatic bile ducts, leading to sclerosing cholangitis.

A study of 57 bone marrow samples from LCH patients showed that there was no significant difference between LCH cases and controls in terms of cellularity, number of monocytes and progenitor cells, and presence of histiocytes and hemophagocytosis (10). In other words, the bone marrow is usually minimally involved.

The pulmonary lesion is characterized by a granulomatous lesion centered around the distal bronchioles with resultant destruction of the airway walls (11,12). These lesions are usually focal and are scattered in the background of normal lung parenchyma. The granuloma is composed of a large number of Langerhans cells in the core surrounded by lymphocytes, eosinophils, neutrophils, plasma

cells, fibroblasts, and pigmented alveolar macrophages. Cavitating lesions are often seen as a result of destruction of the bronchial wall and not secondary to necrosis. In later stages, Langerhans cells gradually decrease and fibrosis takes place as the lesion starts to heal. The fibrotic process may induce retraction emphysema in some cases.

Immunophenotyping

The major markers for the diagnosis of LCH are CD1a, CD207 (Langerin), and S100. CD1a is a reliable marker but is positive for rare cases of juvenile xanthogranuloma (4,6–8). Langerin is a relatively new and specific marker, which is against a type II transmembrane protein associated with Birbeck granules (13). S100 is not as specific because it is positive in most of the dendritic cells as well as nondendritic histiocytes (8). Other markers that are helpful in the distinction of various histiocytic neoplasms include CD68, CD21, CD35, and lysozyme (Table 6.49.1). CD21 and CD35 are specific for follicular dendritic cell neoplasms.

In addition, Langerhans cells also express myelomonocytic markers (CD13, CD33, CD11b, CD11c), CD40, vimentin and HLA-DR (6,14). Adhesion molecules, including CD2, CD31, CD54, and CD58, are also frequently expressed on LCH cells (7), but they play no important roles in the diagnosis of LCH. There are two markers, CD14 and CD52, which are present only on LCH cells but not on normal Langerhans cells (15). Some markers, such as CD80 and CD86, can be demonstrated in LCH cells in certain organs, such as pulmonary LCH (11), but not in other organs, such as bone LCH (16).

Comparison of Flow Cytometry and Immunohistochemistry

Flow cytometry can demonstrate the specific CD1a marker and some myelomonocytic markers, including CD13, CD33, CD11b, and CD11c. However, Langerhans cells may be scattered and low in number, so that it is not an ideal means for the diagnosis of LCH. On the other hand, immunohistochemistry may show all the important markers, such as CD1a, Langerin, and S100, with direct morphologic correlation to the tumor cells and is thus a more ideal tool for the diagnosis.

In the current case, the lesional cells showed characteristic grooved, folded, and indented nuclei. Immunohistochemistry demonstrated that the tumor cells were immunoreactive with CD1a, S100, CD68, and vimentin. These findings are diagnostic of LCH. This case occurred in a young child with bone involvement in two sites and is thus a single-system, multiple site disease, previously

TABLE 6.49.1

Immunophenotypic Differences of Various Dendritic and Histiocytic Neoplasms

	CD1a	CD207	S100	CD68	Lysozyme	CD21/CD35
Langerhans cell neoplasms	+	+	+	+	±	–
Interdigitating cell neoplasms	–	–	+	±	–	–
Follicular dendritic cell neoplasms	–	–	±	±	–	+
Histiocytic sarcoma	–	–	±	+	+	–

called Hand—Schüller—Christian disease. The salient features for laboratory diagnosis of Langerhans cell neoplasm are summarized in Table 6.49.1.

Molecular Genetics

DNA analysis has shown that most cases of LCH have diploid DNA. One study found that only 8% of LCH cases involving lymph nodes revealed aneuploidy (7). No monoclonality has been demonstrated by immunoglobulin heavy-chain gene and T-cell receptor gene rearrangements in LCH cases, except in rare cases where LCH lesions showed the same gene rearrangement as the coexistent B-cell or T-cell lymphoma (7). However, monoclonality has been demonstrated by x-linked androgen receptor gene assay (HUMARA) in most LCH cases, in both localized and disseminated forms, involving lymph node, bone, skin, soft tissue, and bone marrow (6,7).

Using comparative genomic hybridization (CGH), recurrent abnormalities were found in all seven LCH bone lesions, including losses of DNA sequences or gain of DNA copies in various chromosomes (17). Loss of heterozygosity has also been demonstrated in some LCH cases and may represent deletion of tumor suppressor genes (17–19). Overexpression of oncogene proteins, including c-myc, H-ras, bcl-2, Ki-67, RB, TGF- β receptor 1 and 2, MDM2, p53, p21, p16, has also been documented in some cases of LCH (15,17). However, in the studies of pulmonary LCH cases, monoclonal and polyclonal lesions have been demonstrated in the same patients, and it is suggested LCH may begin as a reactive process and gradually evolve into monoclonal lesions (20). Nevertheless, a recent study of large series by multiple techniques, including flow cytometry for DNA ploidy, karyotyping, array CGH, and single nucleotide polymorphism arrays, showed no genomic aberrations in all cases of LCH (21). The salient features for laboratory diagnosis of Langerhans cell neoplasms are summarized in Table 6.49.2.

Clinical Manifestation

Clinically, LCH can be classified into single-system and multisystem disease; the former is further divided into single site and multiple site (Table 6.49.3) (6,16). The

single-system single site disease was previously called solitary eosinophilic granuloma; single-system multiple site disease, Hand—Schüller—Christian disease; and multi-system disease, Letterer—Siwe disease. The single-system single site disease usually involves the bone and less commonly, the lymph node, skin, or lung. The single-system multiple site disease almost always involves the bone. The multisystem disease may involve the bones, skin, liver, spleen, and lymph nodes. Cervical lymph nodes are most often affected. A full-blown hemophagocytic syndrome is usually seen in the multisystem disease. LCH is mainly a pediatric disease, but the single-system single site disease can be seen in both old children and adults. LCH is a rare disease with an estimated incidence of one to two cases per million population (15).

The most common form of LCH is single-system unifocal bony disease and the skull vault is the most common site of involvement, resulting in a painless lump in young children (1,15,22). If the orbit is involved, the patient may present with proptosis. A mastoid lesion may present with swelling and chronic aural discharge. Loose teeth is the sign of mandible involvement. When the base of skull is involved, it may cause damage to the pituitary, leading to diabetes insipidus. Skull bone lesion may also induce neurologic diseases.

Cutaneous lesion is present in about 50% of LCH cases and about 10% are skin-only (1,15,22,23). Cutaneous LH is characterized by a widespread eczematous rash or seborrhea-like eruption, which may or may not be purpuric. Other presentations include papules, vesicles, crusted plaques, nodules, and purpuric nodules. Patients with skin-only LCH may have spontaneous regression and reactivation similar to Hashimoto—Pritzker disease (congenital self-healing reticulohistiocytosis). The axillae, groins, genitalia, or perineum may be severely affected and ulceration may occur. In the mouth, the patient may have swollen gums or ulcers along the cheeks, roof of the mouth, or tongue.

In pulmonary LCH, about two thirds of patients may present with respiratory symptoms (11,12,14). Most patients are smokers. The predominant clinical presentation includes dry cough, dyspnea, and chest pain. Chest pain is frequently associated with pneumothorax, resulting from rupture of a subpleural cystic lesion or with rib involvement in a minority of cases. Wheezing and hemoptysis are rare symptoms. With disease progression, clubbing and cor pulmonale may finally emerge. Physical examination of the chest is usually unremarkable, while blood gas analysis is a more sensitive tool. High-resolution computed tomography is most reliable to detect LCH in the lung, which are nodular lesions with a centrilobular distribution and are frequently accompanied with thin- and thick-walled cystic lesions.

In the central nervous system, hypothalamic-pituitary axis disease is the major clinical manifestation (1,15,22). As a result, 24% of patients may have diabetes insipidus. At the end stage, patients may progress into chronic neurodegeneration due to demyelination and gliosis. Clinical symptoms include cerebellar dysfunction, psychomotor retardation, and neuropsychologic problems.

Gastrointestinal LCH cases may be manifested as hematochezia, nonbloody diarrhea, perianal fistulas, and

TABLE 6.49.2

Salient Features for Laboratory Diagnosis of Langerhans Cell Neoplasms

1. Presence of typical or atypical Langerhans cells.
2. Expression of CD1a, CD207, and S100.
3. Demonstration of Birbeck granules by electron microscopy.
4. No immunoglobulin heavy-chain gene or T-cell receptor gene rearrangements.
5. No nonrandom aberrations in cytogenetic karyotype.

TABLE 6.49.3

Classification of Langerhans Cell Histiocytosis

	Organ involvement	Age group	Synonym
Single-system disease			
Single site (unifocal)	Bone, lymph node, skin, lung	Older children and adults	Solitary eosinophilic granuloma
Multiple site (multifocal)	Bone	Younger children	Hand-Schüller-Christian disease
Multisystem disease	Bone, skin, liver, spleen, lymph node	Infants	Letterer-Siwe disease

constipation (24). In the liver, sclerosing cholangitis may develop as a result of bile duct infiltration by LCH cells and may end up with biliary cirrhosis in chronic cases (1).

The prognosis of LCH varies depending on the clinical forms. The single-system disease carries good prognosis, requiring minimal or no treatment. On the other hand, the multisystem form may be presented as a disseminated life-threatening disease. Patients with involvement of liver, spleen, lungs, or hematopoietic system are considered to be in the high-risk group; while those with skin, bone, lymph node, or pituitary involvement are in the low-risk group (15,25).

REFERENCES

- Windebank KP, Nanduri V. Langerhans cell histiocytosis. *Arch Dis Child*. 2009;94(11):904–908.
- Lichtenstein L. Histiocytosis X: integration of eosinophilic granuloma of the bone, Letterer Siwe disease and Schüller-Christian disease as related manifestations of a single nosologic entity. *AMA Arch Pathol*. 1953;56:84–102.
- Nezelof C, Basset F. From histiocytosis X to Langerhans cell histiocytosis: a personal account. *Int J Surg Pathol*. 2001;9:137–146.
- Favara BE, Feller AC. Contemporary classification of histiocytic disorders. *Med Pediatr Oncol*. 1997;29:157–166.
- Lipscomb MF, Masten BJ. Dendritic cells: Immune regulators in health and disease. *Physiol Rev*. 2002;82:97–130.
- Jaffe R, Weiss LM, Facchetti F. Tumours derived from Langerhans cells. In: Swerdlow SH, Campo E, Harris NL, et al., eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*. Lyon, France: IARC Press; 2008:358–360.
- Ioachim HL, Medeiros LJ. *Ioachim's Lymph Node Pathology*. 4th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2009:531–544.
- Pilleri SA, Grogan TM, Harris NL, et al. Tumors of histiocytes and accessory dendritic cells: an immunohistochemical approach to classification from the International Lymphoma Study Group based on 61 cases. *Histopathology*. 2002;41:1–29.
- Favara BE, Steele A. Langerhans cell histiocytosis of lymph nodes: a morphological assessment of 43 biopsies. *Pediatr Pathol Lab Med*. 1997;17:769–787.
- Minkov M, Potschger U, Grois N, et al. Bone marrow assessment in Langerhans cell histiocytosis. *Pediatr Blood Cancer*. 2007;49:694–698.
- Tazi A, Soler P, Hance AJ. Adult pulmonary Langerhans' cell histiocytosis. *Thorax*. 2000;55:405–416.
- Vassallo R, Ryu JH, Colby TV, et al. Pulmonary Langerhans' cell histiocytosis. *N Engl J Med*. 2000;342:1969–1978.
- Sholl LM, Hornick JL, Pinjus JL, et al. Immunohistochemical analysis of Langerin in Langerhans cell histiocytosis and pulmonary inflammatory and infectious diseases. *Am J Surg Pathol*. 2007;31:947–952.
- Sundar KM, Gosselin MV, Chung HL, et al. Pulmonary Langerhans cell histiocytosis. *Chest*. 2003;123:1673–1683.
- Blesa JMG, Candel VA, Vercet CS, et al. Langerhans cell histiocytosis. *Clin Transl Oncol*. 2008;10:688–696.
- Stockschlaeder M, Sucker C. Adult Langerhans cell histiocytosis. *Eur J Haematol*. 2006;76:363–368.
- Murakami I, Gogusev J, Fournet JC, et al. Detection of molecular cytogenetic aberrations in Langerhans cell histiocytosis of bone. *Hum Pathol*. 2002;33:555–560.
- Dacic S, Trusky C, Bakker A, et al. Genotypic analysis of pulmonary Langerhans cell histiocytosis. *Hum Pathol*. 2003;34:1345–1349.
- Chikwava KR, Hun JL, Mantha GS, et al. Analysis of loss of heterozygosity in single-system and multisystem Langerhans' cell histiocytosis. *Pediatr Develop Pathol*. 2007;10:18–24.
- Yousem SA, Colby TV, Chen YY, et al. Pulmonary Langerhans cell histiocytosis: molecular analysis of clonality. *Am J Surg Pathol*. 2001;25:630–636.
- Da Costa CET, Szuhai K, van Eijk R, et al. No genomic aberrations in Langerhans cell histiocytosis as assessed by diverse molecular technologies. *Gene Chromosome Canc*. 2009;48:239–249.
- Weitzman S, Egeler RM. Langerhans cell histiocytosis: update for the pediatrician. *Curr Opin Pediatr*. 2008;20:23–29.
- Querings K, Starz H, Balda BR. Clinical spectrum of cutaneous Langerhans cell histiocytosis mimicking various diseases. *Acta Derm Venereol*. 2006;86:39–43.
- Hait E, Liang M, Degar B, et al. Gastrointestinal tract involvement in Langerhans cell histiocytosis: Case report and literature review. *Pediatrics*. 2006;118:e1593–e1599.
- Satter EK, High WA. Langerhans cell histiocytosis: a review of the current recommendations of the Histiocyte Society. *Pediatr Dermatol*. 2008;25:291–295.

CASE 50

Thymoma

CASE HISTORY

A 61-year-old man had a resection of the descending colon because of colon cancer 3 months prior to the present admission. In a follow-up computed tomography (CT) scan of the thorax, he was found to have an anterior mediastinal mass, radiologically consistent with a thymoma, and was admitted for removal of the tumor. The patient had no chest pain, cough, dyspnea, superior vena cava syndrome, or signs of myasthenia gravis. Physical examination on admission was unremarkable. At operation, a mass approximately 1.7 cm at its greatest diameter was found in the thymus, and the thymus with the encapsulated

mass was resected en bloc. Postoperatively, he had bilateral pneumothoraces, which were promptly resolved after a chest tube was placed on the right side. The patient was discharged 4 days after the operation.

FLOW CYTOMETRY FINDINGS

Thymectomy specimen: B-cell markers: CD19 0%, k 0%, l 1%. T-cell markers: CD3 19%, CD5 97%, CD7 97%, CD4/CD8 96%. Myeloid markers: CD13-CD33 0%, CD14 0%. Immature cell markers: CD10 48%, CD34 0%, terminal deoxynucleotidyl transferase (TdT) 65% (Fig. 6.50.1).

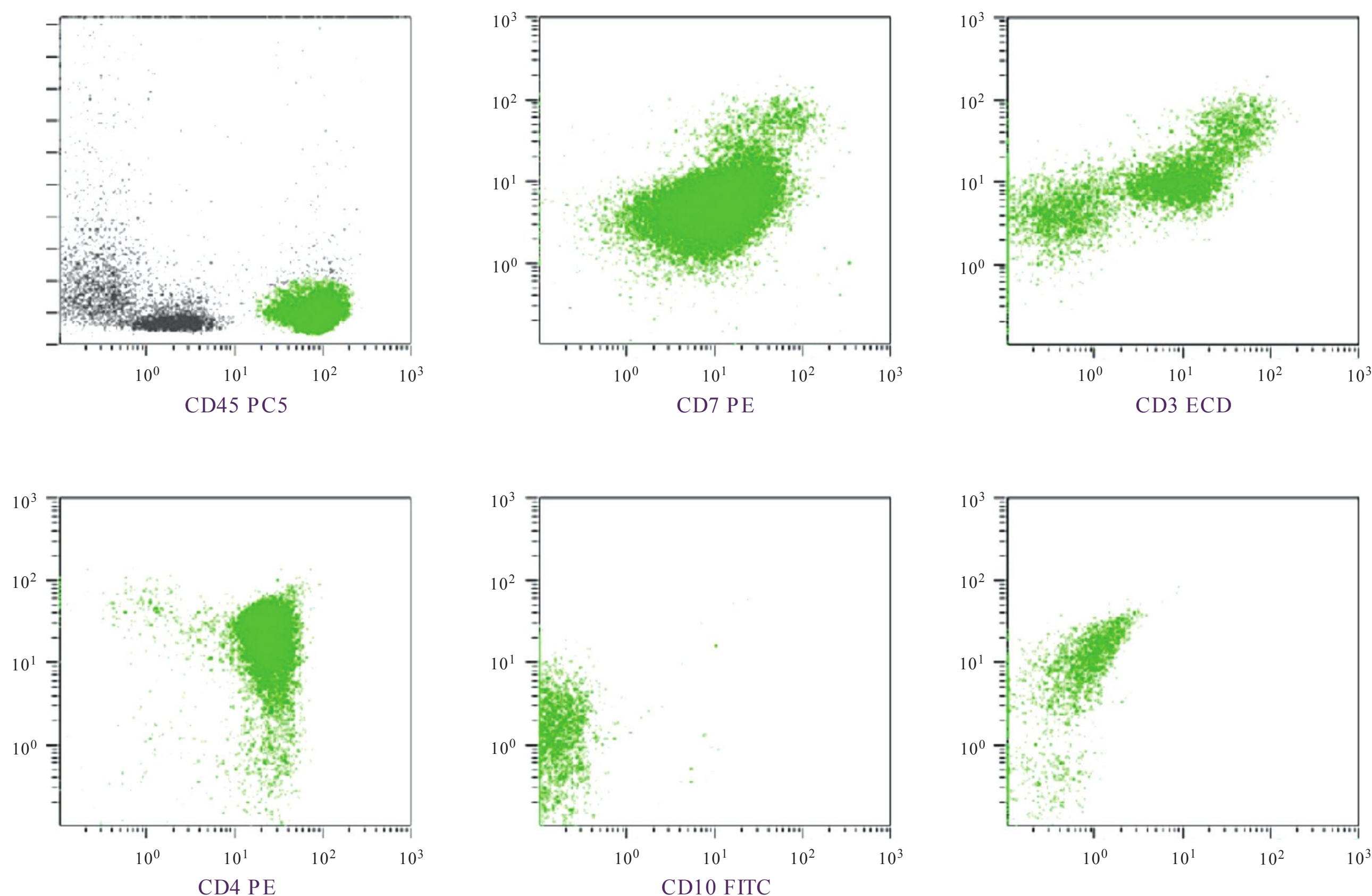


FIGURE 6.50.1 Flow cytometric analysis of a thymus specimen shows positive reactions to CD3, CD4, CD5, CD7, CD8, CD10 (partial), and TdT. The presence of a major population of CD4+ CD8+ cells coexistent with small populations of CD4+ CD8- and CD4- CD8+ cells is characteristic. The smearing pattern of other pan-T-cell markers is also a characteristic feature of thymoma. SS, side scatter; PC5, phycoerythrin-cyanin 5; FITC, fluorescein isothiocyanate; PE, phycoerythrin; ECD, phycoerythrin-Texas Red; RD, rhodamine.

IMMUNOHISTOCHEMICAL STAINS

The lymphoid cells are positive for CD1a, CD3, and CD10. The tumor cells stain for pancytokeratin (Fig. 6.50.2).

MOLECULAR GENETICS

T-cell receptor (TCR) gene rearrangement analysis by polymerase chain reaction shows a germline configuration.

DISCUSSION

Thymoma is an epithelial tumor of the thymus. However, various numbers of lymphocytes are often present and intermingle with the tumor cells. These lymphocytes are, therefore, used as a surrogate marker for the diagnosis of thymoma. The stage of maturation of the lymphocytes also affects the grade of thymoma. The thymic function depends on the interaction between thymocytes and epithelial cells (1). The epithelial tumor cells in a thymoma retain the normal thymic “nursing” function by producing a lymphopoietic environment that attracts uncommitted thymocytes (2,3). Therefore, a small group of thymomas with undifferentiated epithelial tumor cells showed essential absence of lymphocytes (4).

Morphology

Thymoma is characterized by the presence of a lobular pattern separated by broad fibrous bands (5). Each lobule is composed of a dense population of epithelial tumor cells intermingling with varying proportions of lymphocytes or thymocytes. The normal division of cortex and medulla is usually not discernable, except for the organoid thymoma (World Health Organization [WHO] classification type B1) (6). Hassall corpuscles are only seen in organoid thymoma and occasionally in cortical thymoma (WHO classification type B2).

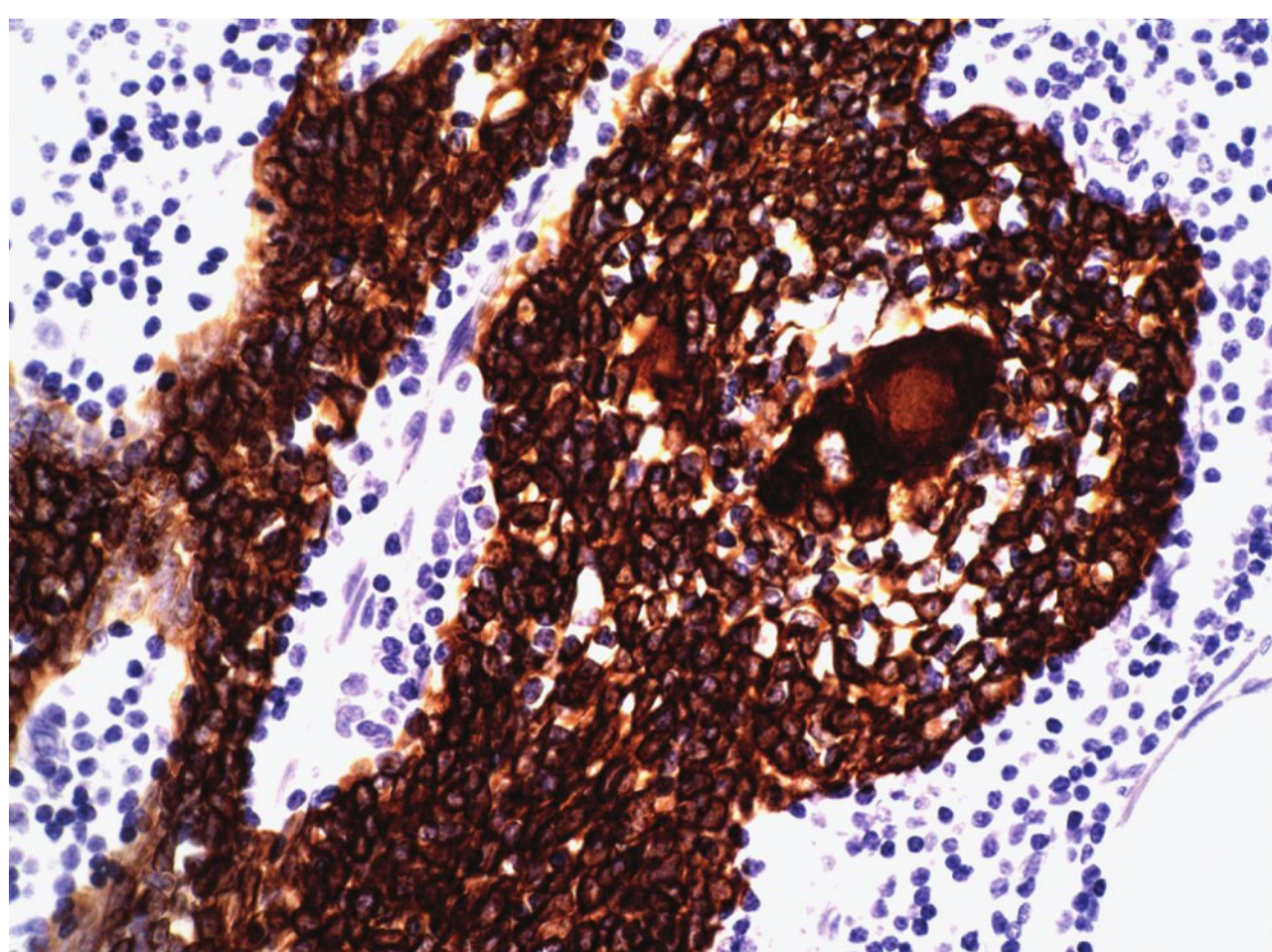


FIGURE 6.50.2 Network of tumor cells stains with cytokeratin, whereas the lymphocytes are negative. Note that a Hassall corpuscle shows strong cytokeratin stain. Immunoperoxidase, 40× magnification.

The epithelial tumor cells are either polygonal (round or oval) or spindle-shaped. The nuclei of the tumor cells are usually vesicular with a small inconspicuous nucleolus. The cytoplasm is of variable amount and pale acidophilic. Most cases contain lymphocytes, but <4% of thymomas may show no lymphocytes at all. Although the immunophenotype of the accompanying lymphocytes may help the diagnosis, it is the identification of the epithelial tumor cells by cytokeratin stain that confirms the diagnosis.

There are many classifications of thymoma. The early classification of Kuo and Lo (7) divides thymomas into predominantly lymphocytic (Fig. 6.50.3), predominantly epithelial (Fig. 6.50.4), mixed lymphocytic and epithelial, and predominantly spindle cells. Levine and Rosai (8) proposed dividing thymomas into benign and malignant on the basis of the presence of capsular invasion. The thymoma with capsular invasion but no prominent cytologic atypia is designated malignant thymoma type I, and those with overt cytologic evidence of malignancy are designated malignant thymoma type II. Marino and Müller-Hermelink (9) introduced a histogenetic classification that divides thymomas into medullary, mixed, predominantly cortical, cortical, and well-differentiated thymic carcinoma. More recently, Suster and Moran (10,11) advocated a simplified classification on the basis of cytologic differentiation. The well-differentiated cytology is designated thymoma; the moderately well-differentiated, designated atypical thymoma; and the poorly differentiated, thymic carcinoma.

Because there is no consensus about the nomenclature of thymic epithelial tumors, the WHO Committee has chosen a noncommittal alphabetic system for classification (Table 6.50.1) (6,12,13). Type A is for tumor cells with a spindle or oval shape, whereas type B is for tumor cells with a dendritic or an epithelioid appearance. Tumors with both features are called type AB. Type B thymomas are further divided into B1, B2, and B3 on the basis of an increasing epithelial cell/lymphocyte ratio and emergence

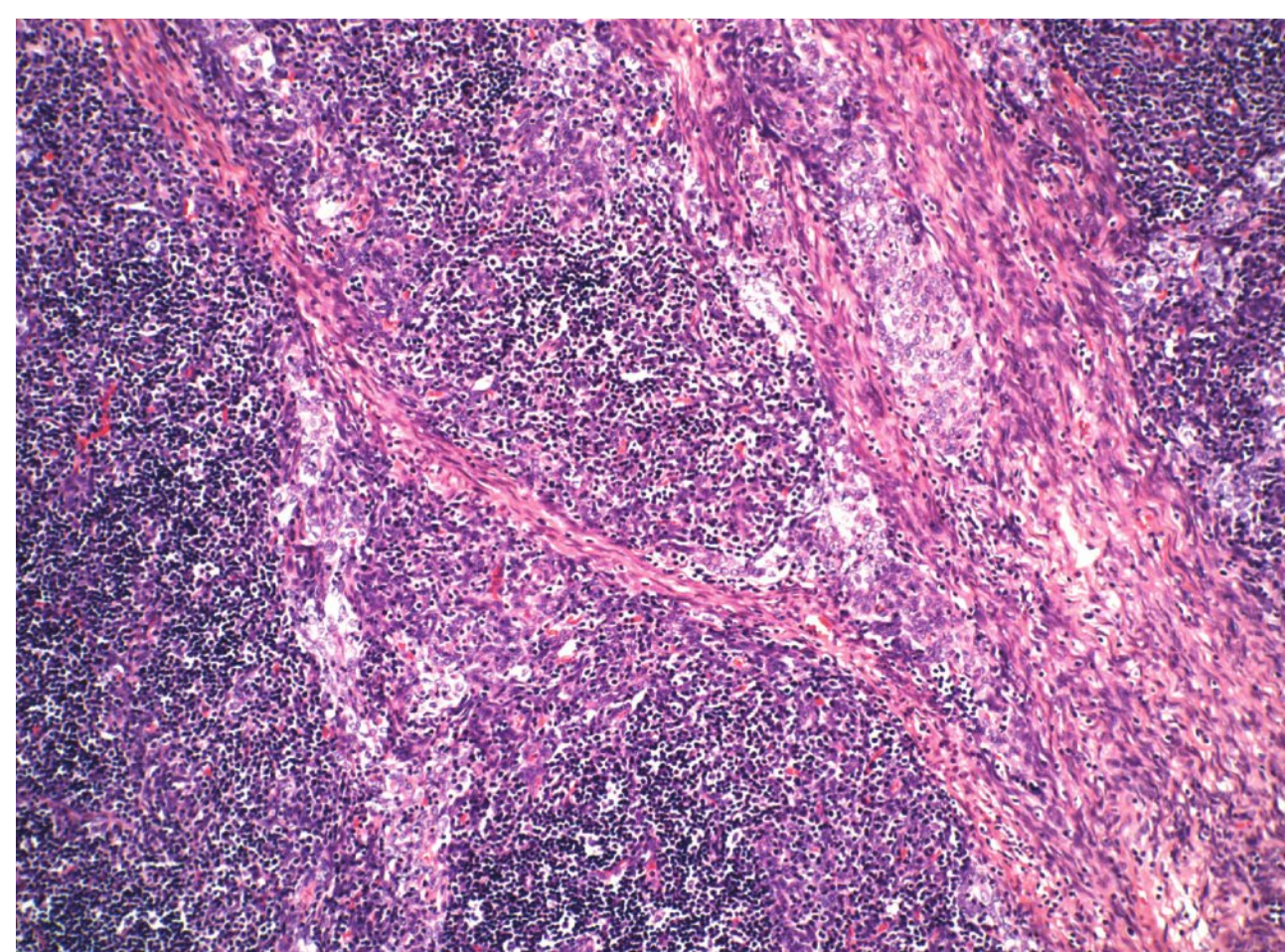


FIGURE 6.50.3 Predominantly lymphocytic thymoma shows a lobular pattern separated by broad fibrous bands. The predominant cell population is lymphocyte with several small clusters of pale-stained epithelial cells. Hematoxylin and eosin, 10 × magnification.

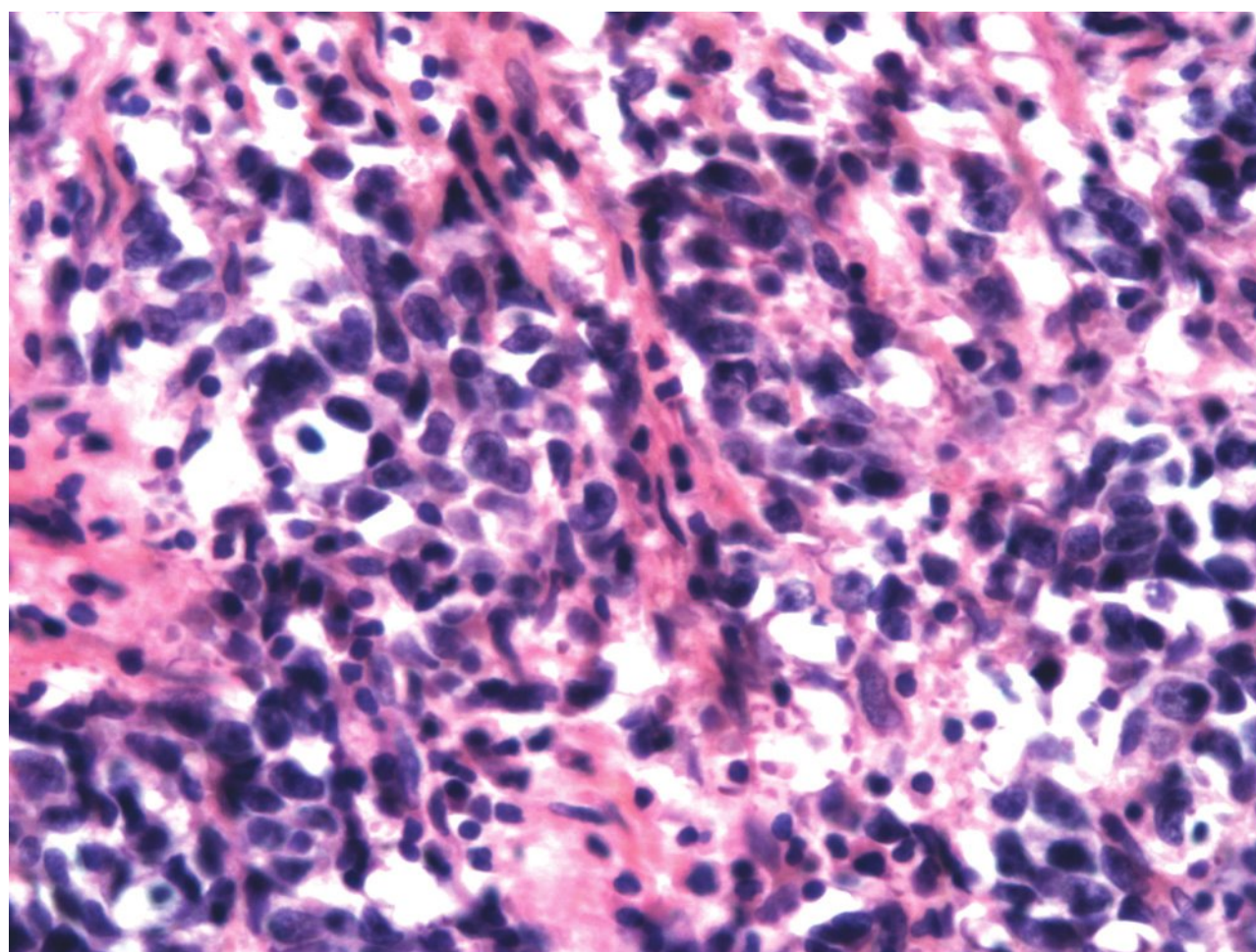


FIGURE 6.50.4 Predominantly epithelial thymoma shows mainly epithelial cells. Hematoxylin and eosin, 60 × magnification.

of atypical neoplastic epithelial cells. Type C is designated for nonorganoid thymic carcinomas.

Type C thymoma is composed of various kinds of carcinomas that are similar to nonthymic carcinomas, except that the thymic carcinoma may be accompanied by mature lymphocytes. These tumors include keratinizing and nonkeratinizing epidermoid (squamous) carcinomas, lymphoepithelioma-like carcinomas, sarcomatoid carcinoma, clear-cell carcinoma, basaloid carcinoma, mucoepidermoid carcinoma, and undifferentiated carcinoma (12). In most studies, epidermoid carcinoma and lymphoepithelioma-like carcinoma are most common (14). In a small European study series, neuroendocrine tumors (carcinoid included) are as common as epidermoid carcinoma (15). However, the WHO scheme excludes neuroendocrine tumors from thymic carcinoma.

There is a rare type of thymoma, which is composed of extensive areas of hyalinized fibroconnective tissue, constituting about 85% to 90% of the tumor mass. It is

designated as either sclerosing thymoma (16) or ancient thymoma (17). This rare type cannot be classified in any of the above schemes, and the diagnosis can be easily missed if only a small mediastinoscopic biopsy is obtained.

Electron microscopy can be helpful in identifying desmosomes and tonofilaments in the epithelial tumor cells (Fig. 6.50.5). However, because epithelial tumor cells can be readily identified by immunohistochemical stain, electron microscopic examination is seldom needed.

Immunophenotype

In normal thymus, thymocytes first develop in the cortex with the first markers CD2 and CD7 (Table 6.50.2) (18). This is called the early cortex stage. After proliferation in the cortex (late cortex stage), thymocytes start to express CD1, CD5, and finally CD3. As the thymocytes traverse the corticomedullary junction, CD4 and CD8 are expressed together on the cell surface. Therefore, late cortical thymocytes are also called common thymocytes. When in the medulla stage, thymocytes differentiate into helper T cells (CD4+ CD8−) and suppressor T cells (CD4− CD8+). At this stage, CD1 disappears.

In normal thymus, 80% of thymocytes are of the late cortical phenotype, 10% are of the early cortical phenotype, and the other 10% are of the medullary phenotype (3). Accordingly, most lymphocyte-predominant thymomas are of the late cortical phenotype (3).

Ichikawa et al. (1) suggested that the higher proportion of immature thymocytes (CD1+ CD3−, or CD4+ CD8+) and the lower proportion of mature thymocytes (CD4+ CD8−/CD4− CD8+, CD1− CD3+) in thymomas than in the normal thymus represent functional deficiency of the epithelial cells in thymoma, which failed to promote further differentiation of immature thymocytes into mature thymocytes. Because the function of a normal thymus also includes the elimination of autoreactive T cells, a functionally deficient thymoma may finally lead to an autoimmune disorder, myasthenia gravis (MG). Despite the predominance of late-cortical-stage thymocytes (CD4+ CD8+) in most thymomas, small populations of CD4+ CD8− and CD4− CD8+ cells are frequently coexistent (19). The mixed population leads to a

TABLE 6.50.1

WHO Classification of Thymoma

WHO Type	Histogenetic Type	Morphologic Description
A	Medullary thymoma	Predominantly spindle cells
AB	Mixed thymoma	Mixed spindle and epithelial cells
B1	Predominantly cortical	Lymphocyte rich, no atypical epithelial cells
B2	Cortical	Higher epithelial cell/lymphocyte ratio, more atypical tumor cells
B3	Well-differentiated thymic carcinoma	Atypical epithelial cells and some immature lymphocytes
C	Nonorganoid carcinoma	Various types of carcinoma

WHO, World Health Organization.

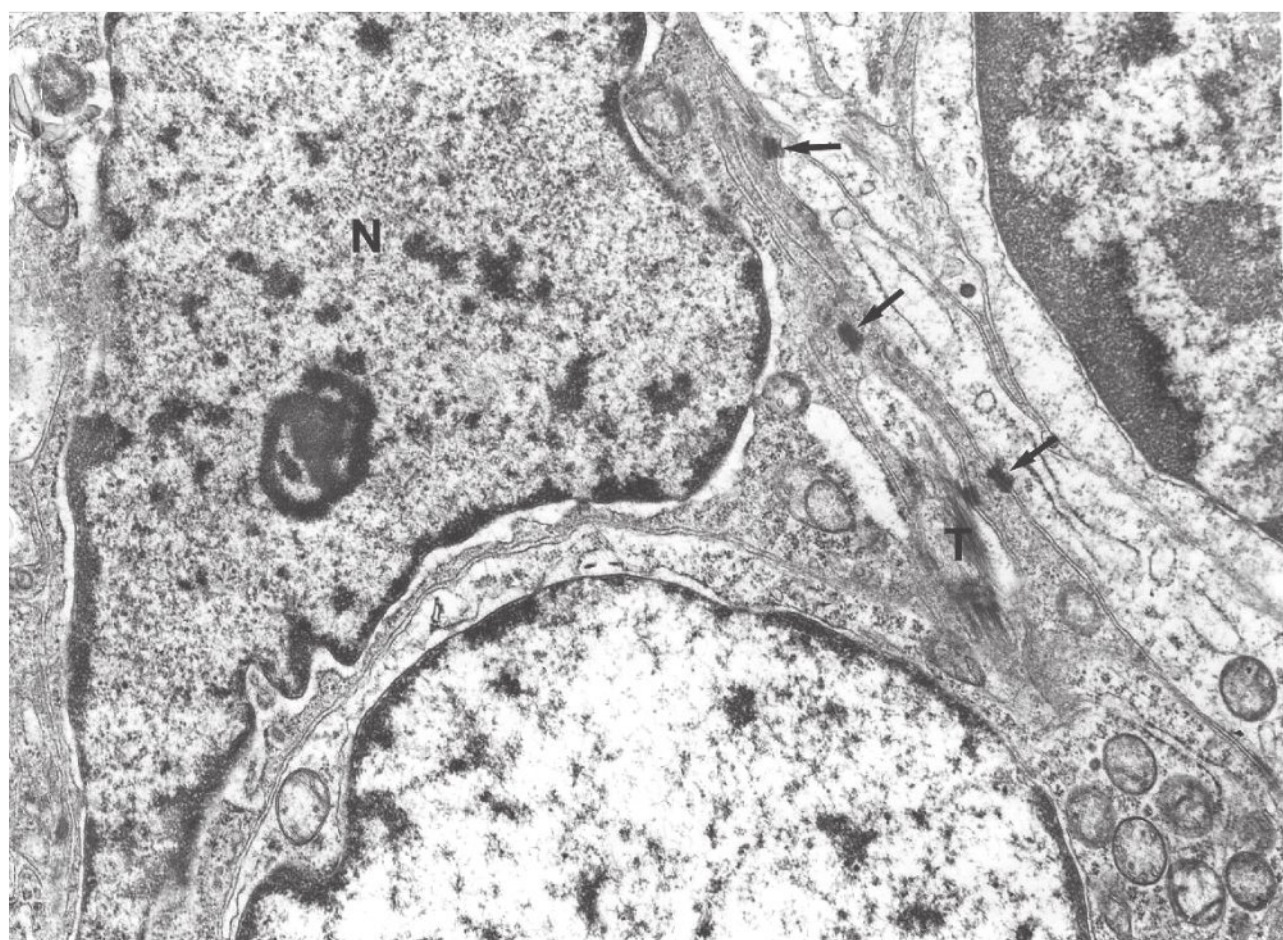


FIGURE 6.50.5 Electron micrograph of a thymoma demonstrates desmosomes (arrows) and bundles of tonofilaments (T). N, nucleus. 23,000 × magnification. (Courtesy of Saul Teichberg, Ph.D., North Shore University Hospital, New York.)

flow cytometric pattern that is highly characteristic of thymoma. In histograms with dual CD45 and a T-cell marker (CD2, CD3, CD5, CD7) staining, three dot clusters are consistently demonstrated. A smear pattern is also characteristically demonstrated with the staining of CD3, CD4, and CD8, due to the presence of heterogeneous populations (19). Another study emphasized the discriminative value of surface CD3 expression versus forward scatter in flow cytometric histograms (20).

These patterns of histogram are very helpful in distinguishing thymoma from lymphoblastic lymphoma. Lymphoblastic lymphoma is the major differential diagnosis, because it is a thymic tumor, frequently located in the mediastinum. The immunophenotypes of these two tumors are very similar. The major differences are that lymphoblastic lymphoma may express CD10 and CD34 and may have selective loss of some T-cell markers (19). Theoretically, thymoma of the early cortical stage may also express CD10 and CD34 (19), but, in reality, this aberrant phenotype is seldom seen. Selective loss of pan-T-cell markers is usually not seen in thymoma. In addition, because lymphoblastic lymphoma is composed of a homogeneous tumor population, the flow cytometric histogram shows a tight cluster pattern instead of a smear pattern as seen in thymomas.

Although CD20+ B cells are frequently found in the medulla of the normal thymus and in areas of medullary differentiation in thymomas by immunohistochemical techniques (20,21), flow cytometry often shows fewer B cells in thymomas than in lymphoblastic lymphoma. Our empirical cutoff point for B cells is 10% in distinguishing these two conditions.

A final diagnosis of thymoma requires immunohistochemical staining. A cytokeratin staining highlighting the network of tumor cells is usually diagnostic. Lymphoblastic lymphoma should be negative for cytokeratin. The presence of CD1a-positive cells in a carcinoma at the site of the thymus denotes thymic carcinoma and distinguishes it from metastatic carcinoma to the thymus (21). However, in a study of 19 cases of thymic carcinoma, the lymphocytes in the tumor were all negative for CD1a, and most cases also showed negative reaction to CD99, indicating that those lymphocytes were in the mature stage (15).

An extensive immunohistochemical study of thymomas showed that cytokeratin was positive in 94% of cases, epithelial membrane antigen in 75%, neuron-specific enolase in 11%, CD57 in 80%, and HLA-DR in 58%, but it was essentially negative for chromogranin and carcinoembryonic antigen (22). However, when neuroendocrine carcinoma was included in the thymoma series, either chromogranin or synaptophysin was demonstrated in these tumor cells (15).

A study of epithelial membrane antigen demonstrated a quantitative difference between thymic carcinoma, invasive thymoma, and noninvasive thymoma; a higher concentration was exhibited in the higher-grade malignancy (23). Additional markers, such as bcl-2 and p53, were also expressed in various kinds of thymic carcinomas (15).

It should be clarified that the negative CD3 reaction in early or common thymocytes is based on the results by flow cytometry, which detects only surface membrane CD3. When frozen sections are examined by immunohistochemical techniques, about 80% of cases of thymoma show cytoplasmic CD3 (24). Furthermore, T-lymphoblastic lymphoma/leukemia may coexist with or transform from thymomas (25,26); this condition makes the distinction between these two tumors more complicated.

Comparison of Flow Cytometry and Immunohistochemistry

Immunohistochemistry is far more helpful than flow cytometry for the diagnosis of thymoma. The cytokeratin stain is most important in defining the epithelial nature of this tumor. However, flow cytometry can help to identify

TABLE 6.50.2								
Immunophenotype of Thymoma at Various Stages								
Stage	TdT	CD1	CD2	CD3	CD4	CD5	CD7	CD8
Early cortex	+	—	+	—	—	—	+	—
Late cortex	+	+	+	±	+	+	+	+
Medulla	+	—	+	+	±	+	+	±

TdT, terminal deoxynucleotidyl transferase

the developmental stage of the thymocyte in the tumor, which may be of clinical significance.

Molecular Genetics

Immunoglobulin and TCR gene analyses in thymomas usually reveal a germline configuration, but germline deletion of the TCR gene may be occasionally encountered (Fig. 6.50.6) (2). Two cases of thymoma were reported to show TCR gene rearrangement for lymphocytes in pleural effusion. Whether this phenomenon represents the malignant nature of these “metastasized” lymphocytes or an aberrant immunologic response to the thymomas is not clear (27).

In recent years, much progress has been made in molecular characterization of thymic tumors, including identification of important oncogenes (EGFR, HER2, KIT, KRAS, and BCL-2), tumor suppressor genes (TP53, p16^{INK4A}), chromosomal aberrations (LOH3p, 6p, 6q, 7p, 8p), angiogenic factors (vascular endothelial growth factor), and tumor invasion factors (matrix metalloproteinases and tissue inhibitor of metalloproteinases) (28).

Epidermal growth factor receptor (EGFR) is overexpressed in thymomas and thymic carcinomas. EGFR gene amplification by fluorescence in situ hybridization (FISH) was detected in 30% of 32 cases of thymomas (29), and EGFR protein overexpression was demonstrated by immunohistochemistry in 46% to 85% of thymomas (30). KIT protein (CD117) is demonstrated by immunostaining in 0% to 5% of thymomas but in 80% of thymic carcinomas (30). In addition, KIT mutation was only seen in thymic carcinomas but not thymomas (30). Therefore, measurement of KIT appears to be helpful in the differential diagnosis between thymomas and thymic carcinoma, and KIT represents a potential target for KIT tyrosine kinase receptor inhibitor.

The histologic thymoma subtypes in the WHO classification show distinct molecular genetic features. With comparative genomic hybridization (CGH) and microsatellite/loss of heterozygosity (LOH) studies, type A and AB thymomas show a low frequency (7% to 8%) of allelic

imbalances, while type B2 and B3 thymomas reveal alterations in about 20% of cases (31).

The most frequently encountered genetic aberrations in all thymic tumors are 6p21.3 (MHC locus) and 6q25.2–25.3 (31). Thymic squamous cell carcinomas frequently show gains of chromosomes 1q, 17q, and 18 and losses of chromosomes 3p, 6, 16q, and 17p (31).

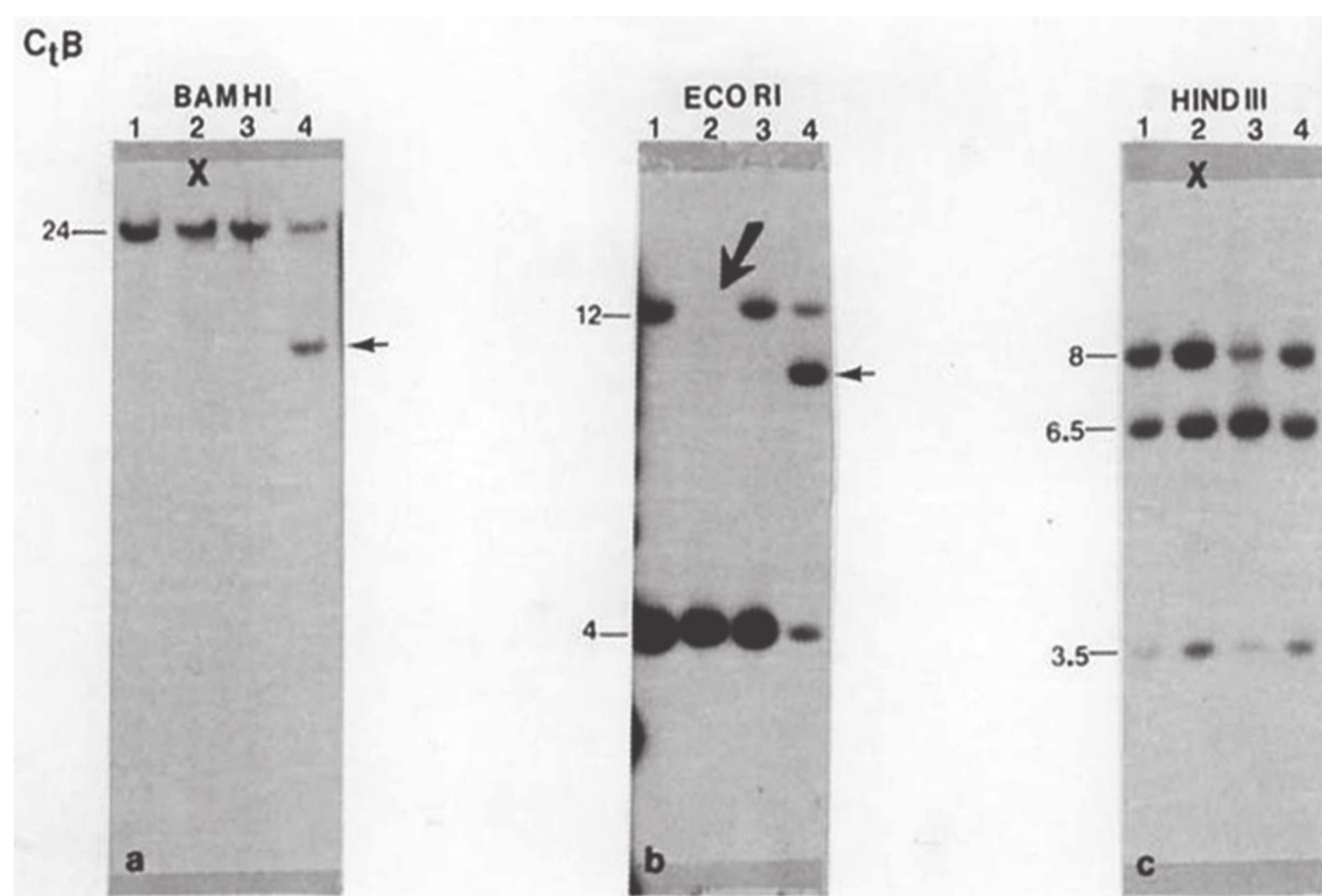
The biopsy of the current case shows an encapsulated mass composed of densely populated lymphoid cells. There are scattered sparsely populated areas in the mass that contain Hassall corpuscles, representing the recapitulation of the thymic medulla (Fig. 6.50.7). A starry-sky appearance is seen in most of the tumor. This appearance, together with the positive staining of CD10 in the lymphocytes, is suggestive of a lymphoblastic lymphoma. However, the presence of Hassall corpuscles, the extensive cytokeratin stain of the epithelial cells, and the flow cytometric pattern confirm the diagnosis of thymoma. Because of the striking resemblance to the normal thymus, this tumor is classified as organoid thymoma or the WHO type B1 thymoma. The salient features of laboratory diagnosis of thymoma are summarized in Table 6.50.3.

Clinical Manifestations

The sex distribution of patients with a thymic tumor is roughly equal, but patients with accompanying MG are more frequently female (18). Although the age of patients ranges from <1 to >90 years, most patients with MG are around the age of 30 to 40 years, and those without MG are around 60 to 70 years old (14). Thymoma patients with MG are associated with better survival than those without MG (32). This is probably due to the work up for MG, resulting in an earlier diagnosis of thymoma. MG is characteristically absent in thymic carcinoma (14).

Approximately one-third of the patients are asymptomatic (14). The symptomatic patients show either local symptoms due to the intrathoracic mass and/or the paraneoplastic (parathymic) syndrome. The chest symptoms include

FIGURE 6.50.6 Southern blot analysis of the TCR b-chain gene (C_B) shows a biallelic deletion of the 12-kb fragments (large arrow) in the thymoma case (lane 2) demonstrated in the EcoRI digest. (From Sun T, Eisenberg A, Benn P, et al. Comparison of phenotyping and genotyping of lymphoid neoplasms. *J Clin Lab Anal.* 1989;3:156–162, with permission).



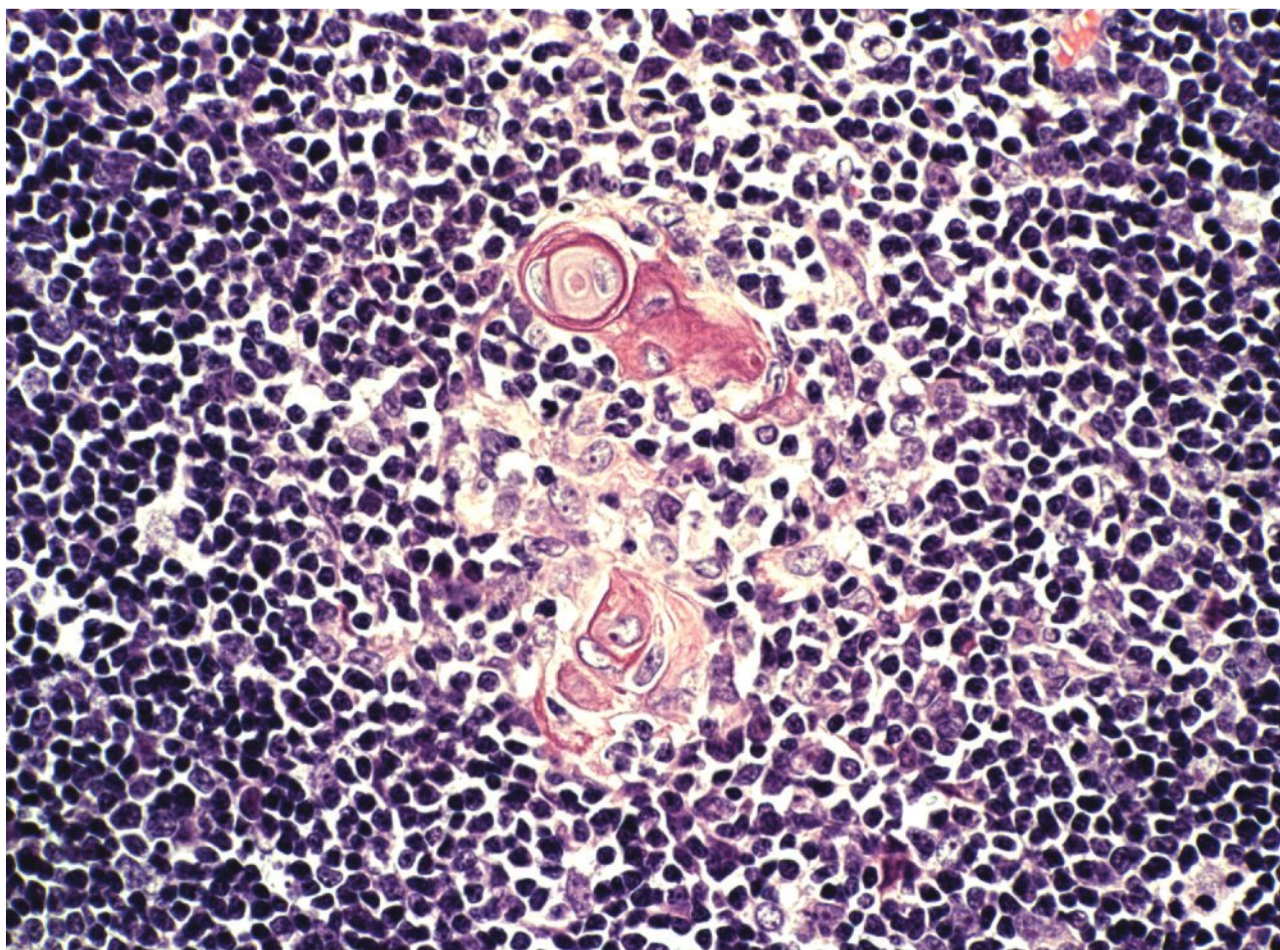


FIGURE 6.50.7 Organoid thymoma shows a pale-stained area that contains Hassall corpuscles, recapitulating the thymic medulla. Hematoxylin and eosin, 20× magnification.

cough, chest pain, dysphagia, dyspnea, hoarseness, and recurrent respiratory infections. Other major clinical manifestations that have been reported include the superior vena cava syndrome, Horner syndrome, cardiac tamponade, disseminated intravascular coagulation, and neck mass (18,33).

There are several parathymic syndromes that are probably autoimmune in nature. The better known entities are MG, with a frequency of about 45%, pure red cell aplasia (2% to 5%), and hypogammaglobulinemia (2% to 5%) (14). Cushing syndrome is only seen in patients with thymic carcinoid tumors (14). Additional conditions that have been associated with thymic tumors, including polymyositis, systemic lupus erythematosus, rheumatoid arthritis, thyroiditis, Graves disease, Sjögren syndrome, and ulcerative colitis, are less

TABLE 6.50.4	
Masaoka Staging System*	
Stage	Definition
I	Macroscopically encapsulated tumor, with no microscopic capsular invasion
Ila	Macroscopic invasion into surrounding fatty tissue or mediastinal pleura
Ilb	Microscopic invasion into the capsule
III	Macroscopic invasion into neighboring organs
IVa	Pleural or pericardial metastases
IVb	Lymphogenous or hematogenous metastasis

*From Masaoka A, Monden Y, Nakahara K, et al. Follow-up study of thymomas with special reference to their clinical stages. *Cancer*. 1981;48:2485–2492.

frequently seen, and their relationship is not fully established (14,34). Several reports also noted the high incidence of secondary malignant tumors in patients with thymoma (14,18).

The prognosis in thymoma patients is mainly related to its stage. The Masaoka staging system is generally accepted as a reliable predictor (Table 6.50.4) (35). Whether the histologic classification is clinically relevant is controversial. Several reports claimed that the WHO scheme is useful clinically (36–38). Two studies concluded that the percentage of invasive tumors was seen in the ascending order in A, AB, B1, B2, B3 and C type tumors (36,38). Other studies considered that the Marino and Müller—Hermelink system is superior in predicting the aggressive potential of thymomas (7,39). In general, the WHO classification is helpful in predicting the prognosis of thymomas (32), but multivariate analyses have shown that the histologic type was not of independent prognostic value (14). All thymic tumors, regardless of their grades, can manifest malignant behavior, namely, recurrence. The long-term survival after complete resection in patients with a stage III or IV thymoma is similar to that in patients with a stage I thymoma (14). Therefore, Detterbeck and Parsons (14) considered that the subclassification of bland tumor is of questionable prognostic value; it is only useful to distinguish thymic carcinoma, well-differentiated thymic carcinoma (atypical thymoma) from bland thymomas (typical thymomas), as Suster and Moran suggested. In the light of recent advances of molecular genetics, a multidisciplinary approach, including clinical manifestation, morphology, and molecular genetics, should be the most reliable way to determine the prognosis.

REFERENCES

1. Ichikawa Y, Shimizu H, Yoshida M, et al. Two color flow cytometric analysis of thymic lymphocytes from patients with myasthenia gravis and/or thymoma. *Clin Immunol Immunopathol*. 1992;62:91–96.

TABLE 6.50.3
Salient Features for Laboratory Diagnosis of Thymoma
1. TdT is positive in all stages.
2. Reactions to T-cell markers (CD1, CD2, CD3, CD4, CD5, CD7, CD8) depend on the stage.
3. Common thymocyte stage (CD1+, CD4+, CD8+) is most common.
4. CD117 (KIT) is positive for thymic carcinoma but negative for thymoma
5. Immunohistochemistry: Positive for cytokeratin and negative for LCA (CD45)
6. Electron microscopy: Presence of desmosomes and tonofilaments
7. TCR gene rearrangement analysis: Germline or deletion

LCA, leukocyte common antigen; TdT, terminal deoxynucleotidyl transferase.

2. Katzin WF, Fishleder AJ, Linden MD, et al. Immunoglobulin and T-cell receptor genes in thymomas: genotype evidence supporting the non-neoplastic nature of the lymphocytic component. *Hum Pathol.* 1988;19:323–328.
3. Gatzimos KR, Mariarty AT, Pingleton JM, et al. Diagnosis of metastatic thymoma using flow cytometry. *Pathobiology.* 1992;60:168–172.
4. Monden Y, Taniolka T, Maeda M, et al. Malignancy and differentiation of neoplastic epithelial cells of thymoma. *J Surg Oncol.* 1986;31:130–138.
5. Rosai J. The pathology of thymic neoplasia. In: Berard CW, Dorfman RF, Kaufman N, eds. *Malignant Lymphoma.* Baltimore: Williams & Wilkins; 1986:161–183.
6. Rosai J, Sobin L. Histological typing of tumours of the thymus. In: World Health Organization International Histological Classification of Tumours. 2nd ed. New York: Springer; 1999:9–14.
7. Kuo TT, Lo SK. Thymoma: a study of the pathologic classification of 71 cases with evaluation of the Müller-Hermelink system. *Hum Pathol.* 1993;24:766–771.
8. Levine GD, Rosai J. Thymic hyperplasia and neoplasia: a review of current concepts. *Hum Pathol.* 1978;9:495–515.
9. Marino M, Müller-Hermelink HK. Thymoma and thymic carcinoma: relation of thymoma epithelial cells to the cortical and medullary differentiation and histologic features. *Virchows Arch.* 1985;407:119–149.
10. Suster S, Moran CA. Primary thymic epithelial neoplasms: spectrum of differentiation and histologic features. *Semin Diagn Pathol.* 1999;16:2–17.
11. Suster S, Moran CA. Thymoma, atypical thymoma, and thymic carcinoma. A novel conceptual approach to the classification of thymic epithelial neoplasms. *Am J Clin Pathol.* 1999;111:826–833.
12. Marx A, Müller-Hermelink HK. From basic immunobiology to the upcoming WHO-classification of tumors of the thymus. The second conference on biological and clinical aspects of thymic epithelial tumors and related recent developments. *Pathol Res Pract.* 1999;195:515–533.
13. Müller-Hermelink HK, Marx A. Pathological aspects of malignant and benign thymic disorders. *Ann Med.* 1999;31(suppl 2):5–14.
14. Detterbeck FC, Parsons AM. Thymic tumors. *Ann Thorac Surg.* 2004;77:1860–1869.
15. Chalabreysse L, Etienne-Mastroianni B, Adeleine P, et al. Thymic carcinoma: a clinicopathological and immunological study of 19 cases. *Histopathology.* 2004;44:367–374.
16. Kuo T. Sclerosing thymoma: a possible phenomenon of regression. *Histopathology.* 1994;25:289–291.
17. Moran CA, Suster S. “Ancient” (sclerosing) thymomas: a clinicopathologic study of 10 cases. *Am J Clin Pathol.* 2004;121:867–871.
18. Morgenthaler TI, Brown IR, Colby TV, et al. Thymoma. *Mayo Clin Proc.* 1993;68:1110–1123.
19. Li S, Luco J, Mann KP, et al. Flow cytometry in the differential diagnosis of lymphocyte-rich thymoma from precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma. *Am J Clin Pathol.* 2004;121:268–274.
20. Gorczyca W, Tugulea S, Liu Z, et al. Flow cytometry in the diagnosis of mediastinal tumors with emphasis on differentiating thymocytes from precursor T-lymphoblastic lymphoma/leukemia. *Leuk Lymphoma.* 2004;45:529–538.
21. Kirchner T, Schalke B, Buchwald J, et al. Well-differentiated thymic carcinoma: an organotypical low-grade carcinoma with relationship to cortical thymoma. *Am J Surg Pathol.* 1992;16:1153–1169.
22. Konstein MJ, Curran WJ Jr, Turrisi AT III, et al. Cortical versus medullary thymomas: a useful morphologic distinction? *Hum Pathol.* 1988;19:1335–1339.
23. Fukai I, Masaoka A, Hashimoto T, et al. The distribution of epithelial membrane antigen in thymic epithelial neoplasms. *Cancer.* 1992;70:2077–2081.
24. Knowles DM. Lymphoblastic lymphoma. In: Knowles DM, ed. *Neoplastic Hematopathology.* 2nd ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2001:915–951.
25. Macon WR, Rynalski TH, Swerdlow SH, et al. T-cell lymphoblastic leukemia/lymphoma presenting in a recurrent thymoma. *Mod Pathol.* 1991;4:525–528.
26. Friedman HD, Inman D, Hutchinson RE, et al. Concurrent invasive thymoma and T-cell lymphoblastic leukemia and lymphoma. A case report with necropsy findings and literature review of thymoma and associated hematologic neoplasm. *Am J Clin Pathol.* 1994;101:432–437.
27. Delannoy A, Philippe M, Hamels J, et al. Clonal rearrangement of the T-cell receptor beta-chain gene in the pleural fluid of a patient with thymoma. *Nouv Rev Fr Hematol.* 1993;35:121–124.
28. Kuhn E, Wistuba H. Molecular pathology of thymic epithelial neoplasms. *Hematol Oncol Clin North Am.* 2008;22:443–455.
29. Ionescu DN, Sasatomi E, Cieply K, et al. Protein expression and gene amplification of epidermal growth factor receptor in thymomas. *Cancer.* 2005;103:630–636.
30. Chau NG, Kim ES, Wistuba I. The multidisciplinary approach to thymoma: Combining molecular and clinical approaches. *J Thorac Oncol.* 2010;5(suppl 4):S313–317.
31. Ströbel P, Hohenberger P, Marx A. Thymoma and thymic carcinoma: molecular pathology and targeted therapy. *J Thorac Oncol.* 2010;5(suppl 4):S286–S290.
32. Venuta F, Anite M, Diso D, et al. Thymoma and thymic carcinoma. *Eur J Cardiothorac Surg.* 2010;37:13–25.
33. Dib HR, Friedman B, Khouli H, et al. Malignant thymoma: a complicated triad of SVC syndrome, cardiac tamponade, and DIC. *Chest.* 1994;105:941–942.
34. Levy Y, Afek A, Sherer Y, et al. Malignant thymoma associated with autoimmune diseases: a retrospective study and review of the literature. *Semin Arthritis Rheum.* 1998;28:73–79.
35. Masaoka A, Monden Y, Nakahara K, et al. Follow-up study of thymomas with special reference to their clinical stages. *Cancer.* 1981;48:2485–2492.
36. Okumura M, Miyoshi S, Fujii Y, et al. Clinical and functional significance of WHO classification on human thymic epithelial neoplasms: a study of 146 consecutive tumors. *Am J Surg Pathol.* 2001;25:103–110.
37. Chalabreysse L, Roy P, Cordier JF, et al. Correlation of the WHO schema for the classification of thymic epithelial neoplasms with prognosis. *Am J Surg Pathol.* 2002;26:1605–1611.
38. Kondo K, Yoshizawa K, Tsuyuguchi M, et al. WHO histologic classification is a prognostic indicator in thymoma. *Ann Thorac Surg.* 2004;77:1183–1188.
39. Vaideeswar P, Padmanabhan A, Deshpande JR, et al. Thymoma: a pathological study of 50 cases. *J Postgrad Med.* 2004;50:94–97.



Note: Page numbers followed by a “f” indicate figures; those followed by a “t” indicate tables.

- A**
- Abnormal localization of immature precursors. See ALIP
- aCML (atypical chronic myeloid leukemia), 89, 90, 90f, 90t, 91t, 93
- Acquired immunodeficiency syndrome. See AIDS
- Activation antigens, 53
- Acute erythroid leukemia. See AML-M6
- Acute leukemia, 41–42, 42t, 43t. See also Specific leukemias
- Acute lymphoblastic leukemia. See ALL
- Acute megakaryoblastic leukemia. See AML-M7
- Acute monoblastic and monocytic leukemia. See AML-M5
- Acute myeloblastic leukemia. See AML with maturation. See AML-M2 without maturation. See AML-M1
- Acute myeloid leukemia. See AML with inv(16)(p 13;q22) or t(16; 16)(p13;q22). See AML, with inv(16) or t 16; 16, case study of with maturation. See AML-M2) minimally differentiated. See AML-M0 with t(8;21)(q22;q22). See AML, with t(8;21), case study of without maturation. See AML-M1
- Acute myelomonocytic leukemia. See AML-M4 with eosinophilia. See AML-M4Eo
- Acute promyelocytic leukemia. See APL with t(15; 17). See AML-M3
- Adhesion molecules, 54–55
- Adult T-cell leukemia/lymphoma. See ATCL
- AIDS (acquired immunodeficiency syndrome), 1, 31
- AITCL (angioimmunoblastic T-cell lymphoma), case study of classification of, 378 clinical manifestations in, 382–383 discussion about, 378–379 FC and IH, comparison of, 382 history of, 378, 379f IH in, 378, 379f, 380f, 381f immunophenotype in, 381–382 molecular genetics in, 382, 382t morphology in, 379–381, 382t
- ALCL (anaplastic large cell lymphoma), case study of classification of, 384, 386 clinical manifestations in ALK-negative primary systemic ALCL, 391 ALK-positive primary systemic ALCL, 391 primary cutaneous anaplastic large cell lymphoma, 391 secondary anaplastic large cell lymphoma, 391 discussion about FC and IH, comparison of, 389 immunophenotype in, 386–388, 386f, 387f, 388f, 389t molecular genetics in, 389–390, 390t morphology in, 384–386, 385f, 386f FC findings in, 384, 384f history of, 383 IH in, 384 molecular genetic findings in, 384 variants of, 385–386
- Algorithmic approach, for antibody selection, 20
- ALIP (abnormal localization of immature precursors), 79–80, 81f, 82f, 84
- ALK (anaplastic large-cell kinase), 395
- ALK-negative primary systemic ALCL, 391
- ALK-positive primary systemic ALCL, 391
- ALL (acute lymphoblastic leukemia), 16. See also B-precursor ALL AML and, comparison of, 176t classification of, 173–174, 175t, 177t
- AMKL (acute megakaryoblastic leukemia). See AML-M7
- AML (acute myeloid leukemia), 16, 25, 28, 33, 35, 78, 109, 160, 161, 174 ALL and, comparison of, 176t
- AML (acute myeloid leukemia), with inv(16) or t(16; 16), case study of classification of, 101, 116, 117t, 127, 131, 138, 173–174 clinical manifestations of, 105 cytochemical findings in, 101, 102f cytogenetic findings in, 102, 102f FC and IH, comparison of, 104 FC findings in, 101, 101f history of, 101 immunophenotype in, 103–104 molecular genetics in, 104–105, 105t morphology and cytochemistry in, 103f, 104–103, 104f
- AML (acute myeloid leukemia), with t(8;21), case study of clinical manifestations in, 99 cytochemical findings in, 95 FC and IH, comparison of, 98 FC findings in, 95, 95f history of, 95 immunophenotype in, 97–98 molecular genetics in, 96, 96f, 98–99, 99t morphology in, 96–97, 97f, 98f, 98t
- AML-M0 (acute myeloid leukemia, minimally differentiated), 43t, 102, 120, 121t, 160
- AML-M1 (acute myeloblastic leukemia, without maturation), 102, 127, 161 case study of clinical manifestations in, 122–123 cytochemical findings in, 115 cytochemistry in, 116–118, 118f FC and IH, comparison of, 121 FC findings in, 115, 116f history of, 115 immunophenotype in, 119–120, 120t molecular genetics in, 121–122, 121t, 123t morphology in, 118–119, 118f, 119f, 119t
- AML-M2 (acute myeloblastic leukemia, with maturation), 96, 99, 161
- AML-M1 relating to, 115–122, 121t, 199t
- AML-M3 relating to, 102 case study of clinical manifestations in, 128–129 cytochemistry in, 126–127, 127f FC findings in, 125, 126f history of, 125 immunophenotype in, 127–128, 128f, 128t molecular genetics in, 125, 128 morphology in, 125–126, 126f, 127f
- AML-M3 (acute promyelocytic leukemia, with t(15: 17)), 139, 161
- AML-M1 relating to, 115–122, 121t, 199t case study of clinical manifestations in, 112–113 cytochemical findings of, 107, 109f FC and IH, comparison of, 110 FC findings of, 107, 108f history of, 107, 107f immunophenotype in, 108–110, 110f molecular genetics in, 110–112, 111f, 111t, 112f, 113t morphology and cytochemistry in, 107–108, 109f FISH relating to, 112, 112f
- AML-M3v (hypogranular or microgranular APL), 108–112, 109, 110, 111
- AML-M4 (acute myelomonocytic leukemia), 127, 161
- AML-M1 relating to, 115–122, 121t, 199t
- AML with inv(16) or t(16;16), 102–105, 113t case study of clinical manifestations in, 133–134 cytochemical findings in, 130 FC findings in, 130, 131f history of, 130, 130f, 131f immunophenotype in, 132–133, 133f molecular genetics in, 133, 133t morphology and cytochemistry in, 132, 132f
- AML-M4Eo (acute myelomonocytic leukemia, with eosinophilia), 102–104, 121t, 122, 132, 161
- AML-M5 (acute monoblastic and monocytic leukemia), 102, 127, 161
- AML-M1 relating to, 115–122, 121t, 199t case study of clinical manifestations in, 139–140, 140f cytochemical findings in, 135, 136f cytogenetic findings in, 135 FC and IH, comparison of, 138, 138f FC findings in, 135, 136f history of, 135 immunophenotype in, 137–138 molecular genetics in, 138–139, 139t morphology in, 135–137, 136f, 137f
- AML-M6 (acute erythroid leukemia), 102
- AML-M1 relating to, 115–122, 121t, 199t



- AML-M6 (acute erythroid leukemia) (Continued)
 case study of
 clinical manifestations in, 146–147
 discussion of, 142, 143t
 FC and IH, comparison of, 145, 145f, 146f
 FC findings in, 141, 142f
 history of, 141
 immunophenotype in, 144–145
 molecular genetics in, 145–146, 146t
 morphology and cytochemistry in, 142–144, 144f
- AML-M7 (acute megakaryoblastic leukemia), 102
- AML-M1 relating to, 115–122, 121t, 199t
 case study of
 clinical manifestations in, 153–154
 cytochemical findings in, 148
 FC and IH, comparison of, 152, 152f
 FC findings in, 148, 149f
 history of, 148
 immunophenotype in, 151–152, 152f
 molecular genetics in, 152–153, 153t
 morphology and cytochemistry in, 149–151, 150f, 151f
- Amplification techniques, 18–19
- Anaplastic large cell lymphoma. See ALCL
- Anaplastic lymphoma kinase-negative primary systemic anaplastic large cell lymphoma. See ALK-negative systemic ALCL
- Anaplastic lymphoma kinase-positive primary systemic anaplastic large cell lymphoma. See ALK-positive systemic ALCL
- Anaplastic variant, in DLBCL morphology, 297–298, 297f, 298f, 298t
- Angioimmunoblastic T-cell lymphoma. See AITCL
- Antibiotics, 10t
- Antibodies
 anti-human lymphocyte, 10t
 monoclonal, 3, 20–23, 21t–22t, 22f, 23f
- Antibody method, direct conjugate labeled, 17, 18f
- Antibody panels, monoclonal, selection of, 53–58, 56t, 57t
- Antibody selection, algorithmic approach for, 20
- Antigen retrieval. See AR
- Antigenic epitopes, 20
- Antigens
 activation, 53
 cell lineage, 52–53
 histocompatibility, 53
 immature cell, 53
 lineage-associated, 53
 proliferation-associated, 55
 selection of, 53
- Anti-human lymphocyte antibodies, 10t
- APL (acute promyelocytic leukemia), 83 with t(15;17). See AML-M3
- AR (antigen retrieval), 18–19
- ATCL (adult T-cell leukemia/lymphoma), case study of
 cytochemical stains in, 346
 discussion about, 346–350
 clinical manifestations in, 348–350, 348t, 349t, 350t
 FC and IH, comparison of, 347
 immunophenotype in, 347
 molecular genetics in, 347–348
 morphology in, 346, 346f, 347f
 FC findings in, 345, 345f
 history of, 345
- ATRA therapy, 110–111
- Atypical chronic myeloid leukemia. See aCML
- B**
- B cells, 39–40, 40t
 differentiation of, intranodal, 40–41, 41f
 lineage of. See CLL
 population of, 52
- B-CLL. See CLL
- BCR-ABL1-Negative MPN (negative myeloproliferative neoplasms), case study of
 classification of, 69
 discussion about
 clinical manifestation in, 75–76, 75f, 76f
 immunophenotype in, 72–73
 molecular genetics in, 73–75
 morphology in, 69–72, 70f, 71f, 72f, 73f, 73t, 74f
 FC findings in, 308, 308f
 history of, 69, 70f
 molecular genetics in, 69
- Biopsy
 of brain, 23f
 of lymph node, 22f, 23f
 of terminal ileum, 23f
- Blastic plasmacytoid dendritic cell neoplasm. See BPDCN
- BL (Burkitt lymphoma/leukemia), case study of
 BLL and, comparison of, 182–183, 183t, 318–327, 320t
 classification of, 319, 321
 clinical manifestations in, 326–327
 discussion about, 320t
 endemic and sporadic, comparison of, 319f
 FC and IH, comparison of, 323
 FC findings in, 39f, 318
 history of, 318
 IH stains in, 318
 immunophenotype in, 322, 324f, 325f
 molecular genetics in, 318, 323–326, 326f, 327t
 morphology in, 319–321, 320f, 320t, 321f, 322f, 323f, 323t, 324f
- BLL (Burkitt-like lymphoma), BL and, comparison of, 182–183, 183t, 318–327, 320t
- B lymphocytes, 40t
- Bortezomib, 236
- BPDCN (blastic plasmacytoid dendritic cell neoplasm), case study of
 discussion about
 clinical manifestations in, 167
 FC and IH, comparison of, 166
 immunophenotype in, 165–166, 166t
 molecular genetics in, 164, 166–167, 167t
 morphology in, 165
 FC in, 164
 history of, 163, 163f, 164f
 IH in, 163
 laboratory diagnosis, 167t
- B-PLL, 215
- B-precursor ALL (precursor B-lymphoblastic leukemia/lymphoma), case study of
 classification of, 173–174
 clinical manifestations in, 179–180
 cytochemical stains in, 173–174
 discussion about, 173, 174f
 FC and IH, comparison of, 178
 FC findings in, 173, 174f
 history of, 173
 immunophenotype in, 175–178, 177t
 molecular genetics in, 178–179, 179t
 morphology and cytochemistry in, 173–175, 175f, 175t, 176f
- Brain biopsy, 23f
- Breakpoint cluster region (BCR)-Ableson (ABL), 69
- Burkitt lymphoma/leukemia. See BL
- Burkitt-like lymphoma. See BLL
- C**
- Case studies
 AITCL, 378–383
 of AML
 with inv(16) or t(16;16), 101–105
 with t(8;21)(q22;q22), 95–99
 of AML-M1, 115–123
 of AML-M2, 125–129
 of AML-M3, 107–113
 of AML-M4, 130–134
 of AML-M5, 135–140
 of AML-M6, 141–147
 of AML-M7, 148–154
 of ATCL, 345–350
 of BL, 318–327
 of BPDCN, 163–167
 of B-precursor ALL, 173–180
 of CLL
 of B-cell lineage, 191–197
 of T-cell lineage, 199–204
 of CML, 60–67
 of DLBCL, 294–300
 of FL, 270–278
 of HCL, 238–245
 of HL, 398–407
 of HSTCL, 352–357
 of IVLBCL, 314–317
 of LCH, 415–419
 of LPL, 218–225, 232, 233
 of MALT lymphoma, 255–262
 of MCL, 285–292
 of MDS, 77–86
 of MDS/MPD, 87–93
 of MF/SS, 358–364
 of MM, 226–236
 of MPAL, 168–172
 MPN, 69–76
 of MS, 156–161
 of NK-cell lymphoma, 336–343
 of NMZL, 264–268
 of PCALCL, 393–397
 of PCFCL, 280–284
 of PMLBCL, 307–312
 of PLL, 212–216
 of PTCL-U, 371–377
 of PTLs, 409–414
 of SLL, 205–210
 of SMZL, 247–254
 SPTCL, 366–370
 of THRBCL, 302–306
 of thymoma, 420–425
 of T-LBL, 181–188
 of T-LGL, 329–334
- Cell antigens, immature, 53
- Cell distinction, parameters for, 4, 8, 12–14, 13f, 13t
- Cell lineage antigens, 52–53

- Cell lineage markers, coexistence of, 52
 Cell markers, immature, 52
 Cell sorter, 8
 Cells. See also Plasma-cell neoplasms;
 TCR; **Specific** case studies
 B cells, 39–42, 40t, 41f, 52
 hematopoietic, 38–40, 39f, 40t
 NK, 39, 203, 330, 332, 354, 375
 physical properties of
 extrinsic, 4
 intrinsic, 4
 plasma, neoplasms of, 2
 reactive, comparison of percentages of,
 13
 T cells, 1, 2, 21, 22
 tumor, 2
 Centroblastic variant, in DLBCL
 morphology, 296–297, 297f
 Cephalosporins, 10t
 CGH (comparative genomic
 hybridization), 396, 418
 Chemotherapeutic agents, 10t
 Childhood myelodysplastic syndrome, 83
 Chromosomal translocations, in
 lymphomas, 30, 32
 Chronic leukemia, 38, 42. See also **Specific**
 leukemias
 Chronic lymphocytic leukemia. See CLL
 Chronic myelogenous leukemia. See CML
 Chronic myelomonocyte leukemia. See
 CMML
 Chronic myeloproliferative disorder. See
 CMPD
 CLA (cutaneous lymphocyte-associated
 antigen), 395
 Classification systems. See EGIL; FAB; Kiel;
 REAL; TUMB; WHO
 Clinical manifestations. See **Specific** case
 studies
 CLL (chronic lymphocytic leukemia), 27f,
 28, 38, 41–43, 213, 238, 288, 289t
 CLL/PLL, PLL and, comparison between,
 193t, 196t, 197t, 212–216
 CLL, of B-cell lineage, case study of
 clinical manifestations in, 196–197, 196t,
 197t
 discussion about, 191
 FC and IH, comparison of, 194–195
 FC findings in, 191, 192f
 history of, 191
 immunophenotype in, 193–194
 molecular genetics in, 195–196, 196f, 196t
 morphology in, 191–193, 192f, 193f,
 193t, 194f
 CLL, of T-cell lineage, case study of
 clinical manifestations in, 204
 discussion about, 200–202
 FC and IH, comparison of, 203
 FC in, 200, 201f
 history of, 199, 200f
 IH in, 199, 200f, 201f
 immunophenotype in, 203
 molecular genetics in, 200, 203, 203t
 morphology in, 202–203, 202t
 CML (chronic myelogenous leukemia),
 case study of
 classification of, 62
 discussion of, 88, 89
 clinical manifestations in, 65–67,
 66t, 67f
 FC and IH, comparison of, 64
 immunophenotyping in, 63–64
 molecular genetics in, 64–65, 65t
 morphology in, 60–63, 61f, 62f, 63f, 64t
 FC findings of, 60
 history of, 60
 molecular genetics of, 60
 CMML (chronic myelomonocyte
 leukemia), 89–91, 90f, 90t, 91t, 92f
 Comparative genomic hybridization. See
 CGH
 Comparison, of FC and IH. See **Specific**
 case studies
 Computer system, of **flow** cytometer
 cell sorter, 8
 contourgram, 8, 8f
 isometric plot, 8, 8f
 scattergram, 7, 7f, 10f
 single histogram, 7, 8f, 11f
 Contourgram, 8, 8f
 Core-binding factor leukemias (CBFLs), 35
 Corticosteroids, 10t
 Coulter Principle, 4
 CTCL (cutaneous T-cell lymphoma), 358,
 359, 364, 364t
 Cutaneous lymphocyte-associated
 antigen. See CLA
 Cutaneous T-cell lymphoma. See CTCL
 Cytochemical **findings**. See **Specific** case
 studies
 Cytochemical stains
 in ATCL case study, 346
 in B-precursor ALL case study, 173–174
 Cytochemistry. See **Specific** case studies
 Cytogenetics, 2, 25–28, 26f, 77, 102, 102f, 135
 array-based whole genome scanning
 technologies, 27–28
 FISH, 26–27, 27f
 Cytogenetic **findings**. See **Specific** case
 studies
 Cytometry. See FC
 Cytomics FC500, 4, 5f
 Cytoplasmic granularity, 12
- D**
 Daunorubicin, 10t
 Dexamethasone, 236
 Diffuse large B-cell lymphoma. See DLBCL
 Direct conjugate-labeled antibody method,
 17, 18f
 DLBCL (diffuse large B-cell lymphoma),
 case study of
 classification of, 295–296
 clinical manifestations in, 300
 discussion about, 295–300
 FC and IH, comparison of, 299
 FC findings in, 295, 295f
 history of, 294–295
 IH stains in, 295, 296f
 immunophenotype in, 298–299, 298f
 molecular genetics in, 295, 299–300, 300t
 morphology in
 anaplastic variant, 297–298, 297f, 298f,
 298t
 centroblastic variant, 296–297, 297f
 immunoblastic variant, 297, 297f
- DNA
 PCR relating to, 30
 RNA and
 contents of, 4, 7, 14
 identification of, 22
 sequences of, 26, 27
 staining of, 6
 transfer of, 29, 30
 Double immunoenzymatic techniques,
 19, 19f, 19t
 Double labeling, 14
- Doxorubicin, 236
 Dysregulated genes, 85, 85t
- E**
 EBV (Epstein-Barr virus), 338, 375, 380,
 386, 396, 405, 410–414
 Electronic system, of **flow** cytometer, 5f,
 6–7
 EMA (Epithelial membrane antigen), 395
 Endemic BL, 320t
 EORTC (European Organization for
 Research and Treatment of Cancer),
 358, 359
 Eosinophilia. See AML-M4Eo
 Eosinophils, 16, 103
 Epigenetics, tumorigenesis, 35
 Epithelial membrane antigen. See EMA
 Epstein-Barr virus. See EBV
 Esterases, 16
 European Organization for Research and
 Treatment of Cancer. See EORTC
 European–American Classification of
 Lymphoid Neoplasms. See REAL
 Extranodal marginal zone B-cell
 lymphoma. See MALT lymphoma, case
 study of
 Extrinsic cells, physical properties of, 4
- F**
 FAB (French–American–British)
 classification, of leukemia, 41–42, 78, 88,
 115, 119t, 121t, 131, 173–174
 FACS (**fluorescence-activated cell-sorting**)
 analyzer, 4
 FACSCanto II, 4, 5f
 FC (**flow** cytometry), 1–2
 advantages of, 1
 cell distinction, parameters for
 cell size, 12, 13t
 cytoplasmic granularity, 12
 DNA and RNA contents, 4, 7, 14
 double labeling, 14
 immunophenotyping, 13, 13f
 intensity of immunofluorescence of
 surface immunoglobulin, 13
 reactive cells, comparison of
 percentages of, 13
 diagnosis of, criteria for
 immunophenotyping of, 1, 2, 3
 subclassification of, 1, 2
 findings See **Specific** case studies)
 fluorescent signals of, 6, 6t
 hematologic neoplasms relating to,
 51–53, 52f, 52t
 IH and, comparison of. See **Specific** case
 studies)
 immunophenotyping relating to, 13, 13f
 limitations of, 1
 results of, factors relating to
 cursor setting, 11, 12f
 FC (**flow** cytometry)
 electronic compensation, 11
 gating, 10–11
 instrument problems, 9–11, 9t, 11f
 reagent problems, 11–12
 specimen problems, 9, 9t, 10f, 10t
 FH (follicular hyperplasia), 270, 272t, 274–275
 FISH (**fluorescence in situ hybridization**),
 23, 23f, 60, 96
 AML-M3 relating to, 112, 112f
 MCL relating to, 285, 291
 molecular genetics relating to, 26–27, 27f

5q-syndrome, 82, 85
 Fixatives, 17
 FL (follicular lymphoma), case study of, 288, 289t
 classification of, 272
 cytogenic findings in, 270
 discussion about, 270
 clinical features in, 278
 FC and IH, comparison of, 275–276, 275f, 276f
 immunophenotype in, 275
 molecular genetics in, 276–278, 277f, 278t
 morphology in, 270–275, 271t, 272t, 273f, 274f, 275f
 FC findings in, 270, 271f
 FH and, 270, 272t, 274–275
 history of, 270
 IH findings in, 270
 Flow cytometer
 Cytomics FC500, 4, 5f
 FACS analyzer, 4
 FACSCanto II, 4, 5f
 with fluorescence detectors, 1, 4
 lasers relating to, 4
 principles of, 4–14
 computer system, 7–8, 7f, 8f
 electronic system, 5f, 6–7
 fluid transport system, 4, 5f
 instrumentation, 4, 5f
 optical system, 4–6, 6f, 6t, 7t
 Flow cytometry. See FC
 Fluid transport system, of flow cytometer, 4, 5f
 Fluorescence detectors, 1, 4
 Fluorescence in situ hybridization. See FISH
 Fluorescence-activated cell-sorting analyzer. See FACS (fluorescence-activated cell-sorting) analyzer
 Fluorescent signals, of FC, 6, 6t
 Fluorochromes, 6, 6t, 7t
 Follicular hyperplasia. See FH
 Follicular lymphoma. See FL
 Follicular variant, in PTCL-U morphology, 373
 French–American–British classification, of leukemia. See FAB classification, of leukemia

G

Gating, 10–11
 Gene expression profiling. See GEP
 Genes. See also Oncogenes
 dysregulated, 85, 85t
 immunoglobulin heavy-chain, somatic mutation of, 29
 rearrangement of
 in lymphoproliferative disorders, 31–32
 TCR, immunoglobulin and, 28–29, 28t, 29f
 Genetics. See Molecular genetics
 GEP (gene expression profiling), 33–34, 34f, 382
 Germinal center, 41

H

Hairy cell leukemia (HCL). See HCL
 Hand–Schüller–Christian disease, 415, 418

HCL (hairy cell leukemia), case study of
 discussion about
 clinical manifestations in, 244–245, 244f, 245f
 FC and IH, comparison of, 242, 243f
 immunophenotype in, 240–242, 242f, 243t
 morphology in, 238–240, 239f, 240f, 240t, 241f
 FC findings in, 238, 239f
 history of, 238
 immunochemistry in, 238
 laboratory diagnosis, 244t
 Hematologic neoplasms
 analysis of T-Cell Receptor-Vb (TCR-Vb) repertoire, 53
 classification of, 38–49
 acute leukemias, 41–42, 42t, 43t
 based on clinical presentation, 41
 chronic leukemias, 42
 leukemia, 38
 lymphoma, 42–44, 44t–49t
 multilineage phenotype relating to, 44
 cytogenetics relating to, 25–28, 26f
 diagnosis of, 1, 2
 FC diagnosis of, criteria for, 52t
 coexistence of two different cell lineage markers on same cell population, 52
 expression of immature cell markers in large number of cells, 52
 immunoglobulin light-chain restriction, 51–52
 loss of surface immunoglobulin in B-cell population, 52
 lymphoma, 3
 selective loss of one or more cell lineage antigens, 52–53
 immunophenotyping of, 10t
 Hematopoietic cells
 developmental stages of, 38–40, 39f, 40t
 intranodal B-cell differentiation, 40–41, 41f
 pregerminal center, germinal center, and postgerminal center lymphomas, 41
 Hepatosplenic T-cell lymphoma. See HSTCL
 Histocompatibility antigens, 53
 HL (Hodgkin lymphoma), case study of
 classification of, 399, 399t
 clinical manifestations in, 407, 407t
 discussion about, 398–407, 399t
 FC and IH, comparison of, 405–406, 406t
 FC findings in, 398
 history of, 398
 immunophenotype in, 403–405, 403f, 404f, 405f
 molecular genetics in, 406–407
 morphology in, 399–403, 400f, 401f, 402f
 differential diagnosis in, 403
 LDCHL, 402–403, 402f
 LRCHL, 401–402, 402f
 MCCHL, 401, 402f
 NLPHL, 399–400, 401f
 NSCHL, 400–401, 402f
 Hodgkin lymphoma. See HL
 HSTCL (hepatosplenic T-cell lymphoma), case study of
 classification of, 352–353
 cytochemistry in, 352
 discussion about, 352–357
 clinical manifestations in, 356–357
 FC and IH, comparison of, 355

immunophenotyping in, 354–355, 355f
 molecular genetics in, 355–356, 356t
 morphology in, 353–354, 353f, 354f, 354t
 FC findings in, 352
 history of, 352
 immunogenotyping in, 352
 HTLV-1, 347, 349, 350
 Hypogranular or microgranular APL. See AML-M3v

I

IH (immunohistochemistry)
 advantages of, 1
 in AITCL case study, 378, 379f, 380f, 381f
 in BPDCN, 163
 clinical application of, 51–58
 in CLL case study, 199, 200f, 201f
 cytochemistry and, in MDS/MPD case study, 88
 definition of, 17
 FC and, comparison of. See Specific case studies
 in FL case study, 270
 in HSTCL case study, 355
 in IVLBCL, 314, 315f
 in LCH case study, 415, 416f, 417f
 limitations of, 2
 in PCALCL case study, 393, 394f, 395f
 in PCFCL, 280, 281f, 282f
 principles of, 16–23
 in PTLDs case study, 409, 410f
 in SPTCL case study, 366
 in THRBCL, 302, 302f, 303f
 IH stains, 156, 355
 in BL case study, 318
 in DLBCL case study, 295, 296f
 in LPL case study, 218
 in MS case study, 156
 in PTCL-U case study, 371
 in SLL case study, 205
 in thymoma case study, 421, 421f
 Ilium, terminal, biopsy of, 23f
 Immature cell antigens, 53
 Immunoblastic lymphomas, 2
 Immunoblastic variant, in DLBCL
 morphology, 297, 297f
 Immunofluorescence, of surface
 immunoglobulin, 13
 Immunofluorescent stain, in PMLBCL case study, 308
 Immunoglobulin
 surface
 in B-cell population, loss of, 52
 immunofluorescence of, 13
 TCR gene rearrangement and, 28–29, 28t, 29f, 333–334, 388
 Immunoglobulin heavy-chain gene, somatic mutation of, 29
 Immunoglobulin light-chain restriction, 51–52
 Immunohistochemical staining procedures
 AR and amplification techniques, 18–19
 direct conjugate-labeled antibody
 method, 17, 18f
 double immunoenzymatic techniques, 19, 19f, 19t
 indirect or sandwich method, 17–18, 18f
 monoclonal antibodies for staining, selective use of, 3, 20–23, 21t–22t, 22f, 23f
 quality control of, 19–20
 Immunohistochemistry. See IH



Immunophenotype/immunophenotyping, 22, 38. See also Specific case studies
 FC relating to, 13, 13f
 of hematologic neoplasms, 1, 2, 3, 10t
 In situ hybridization, 23, 23f
 Indirect or sandwich method, 17–18, 18f
 Instrument problems, 9–10, 9t, 11f
 Instrumentation, of flow cytometer, 4, 5f
 International Society for Cutaneous Lymphomas. See ISCL
 Intranodal B-cell differentiation, 40–41, 41f
 Intravascular large B-cell lymphoma. See IVLBCL
 Intrinsic cells, physical properties of, 4
 ISCL (International Society for Cutaneous Lymphomas), 359
 Isometric plot, 8, 8f
 IVLBCL (intravascular large B-cell lymphoma), case study of
 classification of, 315
 discussion about
 clinical manifestations in, 317
 FC and IH, comparison of, 316
 immunophenotype in, 316
 molecular genetics in, 316, 316t
 morphology in, 315–316
 history of, 314, 314f, 315f
 IH in, 314, 315f

J

JMML (Juvenile myelomonocytic leukemia), 66, 88–90, 90t, 92, 92f, 93
 Juvenile myelomonocytic leukemia. See JMML

K

Kiel classification, 42, 44t, 371, 386

L

Langerhans cell neoplasms. See LCH
 Large granular lymphoproliferative disorder. See LGLD
 Lasers, flow cytometer relating to, 4
 LBL (lymphoblastic lymphoma), 173. See also T-LBL
 LCH (Langerhans cell neoplasms), case study of
 classification of, 415
 clinical manifestations in, 418–419, 419t
 discussion about, 415
 FC and IH, comparison of, 417–418
 history of, 415, 415f, 416f
 IH in, 415, 416f, 417f
 immunophenotype in, 417, 417t
 molecular genetics in, 418, 418t
 morphology in, 416–417
 LDCHL (lymphocyte-depleted classical Hodgkin lymphoma), 402–403, 402f
 Lennert lymphoma. See
 Lymphoepithelioid cell variant, in PTCL-U morphology
 Letterer–Siwe disease, 415, 418
 Leukemia. See also ALL; AML; AML-M1; AML-M2; AML-M3; AML-M4; AML-M5; AML-M6; AML-M7; CLL
 aCML, 89, 90, 90f, 90t, 91t, 93
 acute, 38, 41–42, 42t, 43t
 AML, 16, 25, 28, 33, 35, 78, 109, 160, 161, 174, 176t
 with inv(16) or t(16;16), 101–105
 with t(8;21)(q22;q22), 95–99

AML-M0, 102, 120, 121t, 160
 AML-M4Eo, 102–104, 121t, 122, 132, 161
 APL, 103
 ATCL, 345–350
 BL, 318–327
 B-precursor ALL, 173–180
 chronic, 42
 CML, 60–67
 CMML, 89–91, 90f, 90t, 91t, 92f
 HCL, 238–245
 JMML, 66, 88–90, 90t, 92, 92f, 93
 MPAL, 168–172
 NK-LGL, 338–340
 PLL, 212–216
 T-LGL, 329–334
 LGLD (large granular lymphoproliferative disorder), 330, 331t
 Lineage-associated antigens, 53
 LPL (lymphoplasmacytic lymphoma), case study of
 classification of, 219
 cytogenic study in, 218, 227
 discussion about, 218–225
 clinical manifestations, 223–225, 224f
 FC and IH, comparison of, 222
 immunophenotype, 221–222, 222f
 molecular genetics, 222–223, 223t
 morphology, 220–221, 220f, 221f
 FC findings in, 218, 219f
 history of, 218, 218f
 IH stains in, 218
 LRCHL (lymphocyte-rich classical Hodgkin lymphoma), 401–402, 402f
 Lymph node biopsy, 22f, 23f
 Lymphoblastic lymphoma. See LBL
 Lymphocyte-depleted Hodgkin lymphoma. See LDHL
 Lymphocyte-rich classical Hodgkin lymphoma. See LRCHL
 Lymphocytes. See also CLL; NK-LGL leukemia; NLPHL; SLL
 anti-human, antibodies, 10t
 B, 40t
 T, 38, 39, 40t
 Lymphoepithelioid cell variant, in PTCL-U morphology, 372, 374f, 375f
 Lymphoid markers, 120
 Lymphoid neoplasms, 3, 43, 47t
 Lymphomas, 1
 AITCL, 378–383
 ALCL, 383–391
 ATCL, 345–350
 BL, 318–327
 BLL, 182–183, 183t, 318–327, 320t, 323t
 BPDCN, 163–167
 B-precursor ALL, 173–180
 Burkitt, GEP, 33, 34f
 chromosomal translocations in, 30, 32
 classical Hodgkin, 411
 CTCL, 358, 359, 364, 364t
 DLBCL, 294–300
 FL, 270–278
 HL, 398–407
 HSTCL, 352–357
 immunoblastic, 2
 IVLBCL, 314–317
 LBL, 173
 LCH, 415–419
 LPL, 218–225
 MALT, 255–262
 MBCL, 264
 MCL, 285–292
 NK-cell, 336–343

NMZL, 264–268
 non-Hodgkin, 42–43, 44t, 45t, 274, 371
 PCALCL, 393–397
 PCFCL, 280–284
 PMLBCL, 307–312
 pregerminal center, germinal center, and postgerminal center, 41
 PTCL, 371
 PTCL-U, 371–377
 PTLDs, 409–414
 SLL, 38, 41, 205–210
 SMZL, 247–254
 SPTCL, 366–370
 THRBCL, 302–306
 T-LBL, 181–188
 T/NK, 411
 Lymphomatoid papulosis. See LyP
 Lymphoplasmacytic lymphoma. See LPL
 Lymphoproliferative disorders, 31–32
 LyP (lymphomatoid papulosis), 393

M

MALT (mucosa-associated lymphoid tissue) lymphoma, case study of, 255–262, 268
 discussion about, 256
 clinical manifestations in, 262
 discussion about
 FC and IH, comparison of, 260
 immunophenotype in, 259–260
 morphology in, 256–259, 257f, 258f, 259f, 259t
 FC findings in, 255, 256f
 history of, 255
 molecular genetics in, 260, 260t
 t(1;2)(p22;p12), 260t, 261
 t(1;14)(p22;q32), 260t, 261
 t(3;14)(p14;q32), 260t, 261, 261t
 t(11;18)(q21;q21), 260t, 261
 t(14;18)(q32;q21), 260t, 261
 WHO classification of, 256
 Masaoka staging system, 425, 425t
 MBCL (monocytoid B-cell lymphoma), 264
 MCHL (mixed cellularity classical Hodgkin lymphoma), 401, 402f
 MCL (mantle cell lymphoma), case study of
 classification of, 286
 cytogenetic studies in, 285
 discussion about
 clinical manifestations in, 291–292
 FC and IH, comparison of, 289–290
 immunophenotype in, 288–289, 290f
 molecular genetics in, 290–291, 292t
 morphology in, 285–288, 286f, 287f, 287t, 288f, 289t, 290f
 FC findings in, 285, 286f
 FISH relating to, 285, 291
 history of, 285
 MDS (myelodysplastic syndrome), case study of
 classification of, 78, 88
 clinical manifestation in, 85–86, 86t
 cytogenic findings of, 77
 discussion about, 77–86, 88
 dysregulated genes in, 85, 85t
 FC and IH, comparison of, 84
 FC findings of, 77, 78f
 history of, 77
 immunophenotype in, 83–84
 molecular genetics in, 83t
 5q-syndrome, 85
 del(17p) in, 85

- MDS (myelodysplastic syndrome), case study of (Continued)
 inv(3)(q21-26) in, 85
 monosomy 7 syndrome of childhood, 85
 morphology of, 79–83, 79t, 80f, 81f, 82f, 83t
 childhood MDS, 83
 MDS associated with 5q-syndrome, 82
 RA in, 80–81, 84–86
 RAEB, 82–85
 RARS, 82, 84–86
 RCMD, 82, 83, 85, 86
 RCUD, 80
 RN, 81
 RT, 81–82
 unclassifiable MDS, 83, 83t
- MDS/MPD (myelodysplastic/myeloproliferative disease), case study of
 discussion about
 clinical manifestations in, 93
 FC and IH, comparison between, 92
 immunophenotype and cytochemistry, 91–92, 92f
 molecular genetics in, 92–93
 morphology in, 89–91, 89f, 89t, 90f, 90t, 91t
 FC findings of, 87, 88f
 history of, 87
 IH and cytochemistry of, 91–92
 molecular genetic findings of, 88
- Mediastinal (thymic) large B-cell lymphoma. See PMLBCL
- Melphalan, 236
- Methyl-binding domain proteins (MBDs), 35
- MF/SS (mycosis fungoides/Sézary syndrome), case study of
 classification of, 358
 discussion about
 clinical manifestations in, 363–364, 364t
 FC and IH, comparison of, 362
 immunophenotype in, 361–362
 molecular genetics in, 362–363, 363t
 morphology in, 359–361, 360f, 361f, 361t
 FC findings in, 358, 359f
 history of, 358
 IH findings in, 358
- Microgranular APL. See AML-M3v
- MicroRNA (miRNA) alterations, 35
- Mixed cellularity Hodgkin lymphoma. See MCHL
- Mixed phenotype acute leukemia. See MPAL
- MM (plasma cell myeloma) and plasmacytoma, case study of
 classification of, 227t, 229, 233–234
 cytogenic study in, 227
 diagnosis of
 laboratory, 234t
 by WHO, 227t, 234, 235t
 discussion about, 227t
 clinical manifestations in, 234–236, 234f, 235f, 235t, 236f, 236t
 FC and IH, comparison of, 233
 immunophenotype in, 231–233, 232f
 molecular genetics in, 233–234, 234t
 morphology in, 228–231, 228f, 229f, 230f, 231f
 FC findings in, 226
 history of, 226
 plasmablastic type of, 229
 staging systems of, 235t
- Molecular biology, 2, 38
 gene rearrangement in
 lymphoproliferative disorders, 31–32
 GEP, 33–34, 34f
 immunoglobulin and TCR gene rearrangement, 28–29, 28t, 29f, 333–334, 388
 oncogenes, 32–33, 32t
 PCR, 30–31, 30f, 31f
 somatic mutation of immunoglobulin heavy-chain gene, 29
 southern blotting, 29–30, 29f
 tumorigenesis
 epigenetics in, 35
 and signal transduction pathways, 34–35
- Molecular genetics, 3, 25–35. See also Specific case studies
 cytogenetics, 25–28, 26f
 FISH, 26–27, 27f
 as markers for treatment or prognosis, 25
 molecular biology relating to, 2, 28–35, 28t, 29f, 30f, 31f, 32t, 34f, 38
 role of, 25
- Molecules, adhesion, 54–55
- Monoclonal antibodies, 3, 20–23, 21t–22t, 22f, 23f
- Monoclonal antibody panels, selection of, 53–58, 56t, 57t
 adhesion molecules, 54–55
 antigens, 53, 55
 approach
 targeted, 57–58
 two-tiered, 55, 57
 standard panel, 55
- Monocytoid B-cell lymphoma. See MBCL
- Monosomy 7 syndrome, of childhood, 85
- Morphology. See Specific case studies
- MPAL (mixed phenotype acute leukemia), case study of
 clinical manifestations, 172
 discussion about, 169–170
 FC and IH, comparison of, 171
 FC in, 168, 170f
 history of, 168, 169f
 immunophenotype in, 170–171, 171t
 laboratory diagnosis, 172t
 molecular genetics in, 169, 171–172, 172t
 morphology in, 170
- MPNs (myeloproliferative neoplasms), 88
- MPO (myeloperoxidase), 16, 107, 108, 117, 117t, 143, 156
- mRNA (messenger RNA), 28
- MS (myeloid sarcoma), case study of
 classification of, 157
 clinical manifestations in, 161
 cytogenic findings in, 156
 discussion about, 156–157, 157f
 FC and IH, comparison of, 160
 FC findings in, 156, 156f
 history of, 156
 IH stains in, 156
 immunophenotype in, 158–160, 159f, 160f
 molecular genetics in, 160–161, 161t
 morphology in, 157–158, 157f, 158f, 158t
- Mucosa-associated lymphoid tissue lymphoma. See MALT lymphoma, case study of
- Multilineage phenotype, 44
- Multiple myeloma. See MM
- Multiple myeloma oncogene 1. See MUM1
- MUM1 (multiple myeloma oncogene 1), 395
- Mycosis fungoides/Sézary syndrome. See MF/SS
- Myelodysplastic syndrome. See MDS
- Myelodysplastic/myeloproliferative diseases. See MDS/MPD
- Myeloid sarcoma. See MS
- Myeloperoxidase. See MPO
- N**
- Natural killer cell lymphoma. See NK-cell lymphoma
- Natural killer cells. See NK cells
- Neoplasms. See also Hematologic neoplasms
 lymphoid, 3, 43, 47t
 plasma cell, 2
- Nicotine, 10t
- NISH (nonradioactive in situ hybridization), 16
- NK (natural killer) cells, 39, 203, 330, 332, 353, 375
- NK large granular lymphocyte leukemia. See NK-LGL leukemia
- NK-cell lymphoma (natural killer cell lymphoma), case study of
 classification of, 337–338
 discussion about, 337–343
 clinical manifestations in, 342–343, 342f
 FC and IH, comparison of, 341
 immunophenotype in, 340–341, 340t, 341t
 molecular genetics in, 341–342, 342t
 morphology in, 338–339, 339f, 340f
 FC in, 336, 337f
 history of, 336, 336f
 IH in, 336, 338f
 molecular genetics in, 337
- NK-LGL leukemia (NK large granular lymphocyte leukemia), 338, 339
- NLPHL (nodular lymphocyte predominance Hodgkin lymphoma), 399–400, 401f
- NMZL (nodal marginal zone B-cell lymphoma), case study of
 discussion about
 clinical manifestations in, 268
 FC and IH, comparison of, 267
 immunophenotype in, 266–267
 molecular genetics in, 267–268, 268t
 morphology in, 264–266, 266f, 266t, 267f
 FC findings in, 264, 265f
 history of, 264
- Nodal marginal zone B-cell lymphoma. See NMZL
- Nodular lymphocyte predominance Hodgkin lymphoma. See NLPHL
- Nodular sclerosis Hodgkin lymphoma. See NSHL
- Non-Hodgkin lymphoma, 42–43, 44t, 45t, 274, 371
- Nonradioactive in situ hybridization. See NISH
- NOTCH1 signaling, 34
- NSHL (nodular sclerosis classical Hodgkin lymphoma), 400–401, 402f
- O**
- Oncogenes, 32–33, 32t
- Oncogenic-suppressor miRNAs, 35
- Optical system, of flow cytometer, 4–6, 5f, 6f, 6t, 7t
 fluorescent signals in, 6, 6t, 7t
 lens, filter, mirror in, 6

- P**
- PAS (Periodic acid-Schiff) stain, 17, 79, 96, 117, 143, 144
- PCALCL (primary cutaneous anaplastic large-cell lymphoma), case study of clinical manifestations in, 397 discussion about, 393 FC and IH, comparison of, 396 history of, 393, 393f, 394f IH in, 393, 394f, 395f immunophenotype in, 395–396, 396t molecular genetics in, 396–397 morphology in, 394–395
- PCFCL (primary cutaneous follicle center lymphoma), case study of classification of, 281 discussion about clinical manifestations in, 283–284 FC and IH, comparison of, 282–283 immunophenotype in, 282, 283t molecular genetics in, 283, 283t morphology in, 281–282 history of, 280, 281f IH in, 280, 281f, 282f molecular genetics in, 280
- PCR (polymerase chain reaction), 30–31, 30f, 31f, 333
- Periodic acid-Schiff stain. See PAS
- Peripheral T-cell lymphoma. See PTCL
- Peripheral T-cell lymphoma, unspecified. See PTCL-U
- Plasma-cell myeloma. See MM
- Plasma-cell neoplasms, 2
- Plasmablastic type of MM, 229
- Plasmacytoma. See MM (plasma-cell myeloma) and plasmacytoma, case study of
- PLL (prolymphocytic leukemia), case study of, 245. See also B-PLL; T-PLL
- CLL/PLL, CLL and, comparison between, 193t, 196t, 197t discussion about clinical manifestations, 216 FC and IH, comparison of, 215 immunophenotype, 215 molecular genetics, 215–216, 216t morphology, 213–215, 213f, 213t, 214f, 215f FC findings of, 212, 212f history of, 212
- PMLBCL (primary mediastinal (thymic) large B-cell lymphoma), case study of classification of, 308 discussion about clinical manifestation in, 312 FC and IH, comparison of, 310 immunophenotype in, 309–310, 310f, 311t, 312f, 312t molecular genetics in, 311–312 morphology in, 308–309, 309f, 310t FC findings in, 308, 308f history of, 307 immunofluorescent stain in, 308
- Polymerase chain reaction. See PCR
- Post-transplant lymphoproliferative disorders. See PTLDs
- Precursor B-lymphoblastic leukemia/lymphoma. See B-precursor ALL
- Precursor T-lymphoblastic leukemia/lymphoma. See T-LBL
- Pregerminal center, germinal center, and postgerminal center lymphomas, 41
- Primary cutaneous anaplastic large cell lymphoma, 391
- Primary cutaneous follicle center lymphoma. See PCFCL
- Proliferation-associated antigens, 55
- Prolymphocyte leukemia. See PLL
- PTCL (peripheral T-cell lymphoma), 371 classification of, 373t
- PTCL-U (peripheral T-cell lymphoma, unspecified), case study of classification of, 371 clinical manifestations in, 376–377 discussion about, 371, 373t FC and IH, comparison of, 375 FC findings in, 371, 372f history of, 371 IH stains in, 371 immunophenotype in, 373–375, 375f molecular genetics in, 375–376, 376t morphology in, 371–372, 373f, 373t, 374f follicular variant, 373 lymphoepithelioid cell variant, 372, 374f, 375f T-cell variant, 372
- PTLDs (post-transplant lymphoproliferative disorders), case study of clinical manifestations in, 414 discussion about, 410 history of, 409, 409f IH in, 409, 410f immunophenotype in, 411–412, 411f, 412f molecular genetics in, 412–413, 413f, 414t morphology in, 410–411 classical Hodgkin lymphoma, 411 early lesions, 411 monomorphic PTLD, 411 polymorphic PTLD, 411
- R**
- RA (refractory anemia), 80–81, 84–86
- RAEB (refractory anemia with excess blasts), 82–85
- RARS (refractory anemia with ringed sideroblasts), 82, 84–86
- RCMD (refractory cytopenia with multilineage dysplasia), 82, 83, 85, 86
- RCUD (refractory cytopenia with unilineage dysplasia), 80–82
- Reactive cells, comparison of percentages of, 13
- Reagent problems, 11–12
- REAL (European-American Classification of Lymphoid Neoplasms), 43, 46t, 88, 182, 295–296
- ALCL classification by, 384, 386
- BL classification by, 319, 321
- HSTCL classification by, 352–353
- NK-cell lymphoma classification by, 337–338
- PTCL-U classification by, 371
- Refractory anemia. See RA
- Refractory anemia with excess blasts. See RAEB
- Refractory anemia with ringed sideroblasts. See RARS
- Refractory cytopenia with multilineage dysplasia. See RCMD
- Refractory cytopenia with unilineage dysplasia. See RCUD
- Refractory neutropenia (RN), 81
- Refractory thrombocytopenia (RT), 81–82
- RNA, 26, 29. See also DNA
- S**
- SALCL (systemic anaplastic large-cell lymphoma), 393, 396t
- Sandwich or indirect method, 17–18, 18f
- Scattergram, 7, 7f, 10f
- Secondary anaplastic large cell lymphoma, 391
- Sezary syndrome, 202. See also MFISS
- Single histogram, 7, 8f, 11f
- SLL (small lymphocytic lymphoma), 38, 41, 288, 289t case study of clinical manifestations in, 209–210 discussion about, 205 FC and IH, comparison of, 208–209, 209t FC findings in, 205, 206f history of, 205 IH findings in, 205 immunophenotyping in, 207–208 molecular genetics in, 209 morphology in, 205–207, 206f, 207f, 207t, 208f
- Small lymphocytic lymphoma. See SLL
- Small noncoding RNA interference, 35
- SMZL (splenic marginal zone lymphoma), case study of discussion about clinical manifestations in, 253–254 FC and IH, comparison of, 252, 252f immunophenotype in, 251–252, 251t, 252t molecular genetics in, 252–253, 253f morphology in, 248–251, 249f, 249t, 250f, 251f FC findings in, 247, 248f history of, 247 laboratory diagnosis of, 252t
- Solitary eosinophilic granuloma, 415, 418
- Somatic mutation of immunoglobulin heavy-chain gene, 29
- Southern blotting, 29–30, 29f, 67–68, 68f
- Specimen problems, 9, 9t, 10f, 10t
- Splenic marginal zone lymphoma. See SMZL
- Sporadic BL, 320t
- SPTCL (subcutaneous panniculitis-like T-cell lymphoma), case study of classification of, 366 discussion about, 366–367 history of, 366, 367f IH in, 366 molecular genetics in, 366 morphology in, 367–368 clinical manifestations in, 369–370 FC and IH, comparison of, 368 immunophenotype in, 368, 369t molecular genetics in, 368–369, 369t
- Staging system
- Ann Arbor for Hodgkin lymphoma, 407, 407t
- Binet for CLL, 197, 197t
- Durie-Salmon for myeloma, 234, 235t
- Masaoka for thymoma, 425, 425t
- Rai for CLL, 196–197, 196t
- TUMB for cutaneous T-cell lymphoma, 364, 364t

Stains. See also IH stains;

Immunohistochemical staining
procedures

cytochemical, 173, 346

DNA and RNA relating to, 6

immunofluorescent, 308

PAS, 17, 79, 96, 117, 143, 144, 174, 176

Subcutaneous panniculitis-like T-cell
lymphoma. See SPTCL

Surface immunoglobulin

in B-cell population, loss of, 52

immunofluorescence of, 13

Systemic anaplastic large-cell lymphoma.
See SALCL

T

t(1;22)(p13;q13), 121t

t(2;5)(p23;q35), 389

t(2;8)(p12;q24), 179t, 323

t(3;14)(q27;q32), 299

t(8;14)(q24;q32), 179t, 323

t(8;16)(p11;p13), 121t, 138

t(8;21)(q22;q22), 96f, 99, 121t

t(8;22)(q24;q11), 179t, 324

t(9;14)(p13;q32), 223t

t(9;22)(q34;q11), 64, 179, 179t

t(11;14)(q13;q32), 233, 290–291

t(15;17)(q22;q12), 110–111, 111f, 121t

t(16;16)(p13;q22), 121t

t(1;2)(p22;p12), 260t, 261

t(1;14)(p22;q32), 186, 187t, 260t, 261

t(3;14)(p14;q32), 260t, 261, 261t

t(11;18)(q21;q21), 260t, 261

t(14;18)(q32;q21), 260t, 261, 276–277,
277f, 299

T lymphocytes, 38, 39, 40t

T-ALL, 182

T-cell large granular lymphocytic
leukemia. See T-LGL

T-cell receptor. See TCR

T-cell variant, in PTCL-U morphology, 372

T-cell/histiocyte-rich large B-cell
lymphoma. See THRBCL

T-cells, 1, 2, 21, 22

lineage of. See CLL

T-CLL. See CLL

TCR (T-cell receptor), 28–29, 216, 337, 352,
362, 375–376

TCR gene rearrangement, immunoglobulin
and, 28–29, 28t, 29f, 333–334, 388

Terminal ileum, biopsy of, 23f

Thalidomide, 236

THRBCL (T-cell/histiocyte-rich large B-cell
lymphoma), case study of

classification of, 302–303

discussion about

clinical manifestations in, 306

FC and IH, comparison of, 304

immunophenotype in, 304, 305t

molecular genetics in, 304–305, 306t

morphology in, 303–304

history of, 302, 302f

IH in, 302, 302f, 303f

Thymoma, case study of

classification of, 421, 422t

discussion about

clinical manifestations in, 424–425, 425t

FC and IH, comparison of, 423–424

immunophenotype in, 422–423, 423t

molecular genetics in, 424, 424f,

425f, 425t

morphology in, 421–422, 421f, 422f, 422t

FC findings in, 420, 420f

history of, 420

IH stains in, 421, 421f

molecular genetics in, 421

T-LBL (precursor T-lymphoblastic
leukemia/lymphoma), case study of

clinical manifestations in, 188

discussion about, 182–184

FC and IH, comparison of, 186

FC findings in, 182, 182f

history of, 181–182

immunophenotype in, 184–186, 185t,
186f

molecular genetics in, 186–188, 187t, 188t

morphology in, 182–184, 183f, 183t, 184f

T-LGL (T-cell large granular lymphocytic
leukemia), case study of

classification of, 331

discussion about

clinical manifestations in, 334

FC and IH, comparison of, 333, 333f

immunophenotype in, 332–333, 340

molecular genetics in, 333–334,

333f, 334t

morphology in, 331–332, 331f, 332f

FC findings in, 330, 330f

history of, 329–330

molecular genetics findings in, 330

T-PLL, 213–216

TUMB classification system, for CTCL,
364, 364t

Tumor cells, 2

Tumorigenesis

epigenetics, 35

signal transduction pathway, 34–35

Tumors, types of, 3

Tumor-suppressor miRNAs, 35

V

Vincristine, 236

W

Waldenstrom macroglobulinemia (WM).
See LPL

WHO (World Health Organization), 1

AITCL classification by, 379

ALCL classification by, 384, 386

ALL classification by, 173, 175t, 177t

AML classification by, 116, 117t, 128,
138, 174

BL classification by, 319, 321

B-precursor ALL classification by, 174

CML classification by, 62

DLBCL classification by, 295–296

FL classification by, 272

hematologic neoplasm classification by,
38–49

HL classification by, 399

HSTCL classification by, 352–353

IVLBCL classification by, 315

LCH classification by, 415

LPL classification by, 219, 220

MALT classification by, 256

MCL classification by, 286

MDS classification by, 78

MDS/MPD classification by, 88

MF/SS classification by, 358

MM classification by, 227t, 229,
233–234

MPN classification by, 69

MS classification by, 157

NK-cell lymphoma classification by,
337

PCALCL classification by, 393

PCFCL classification by, 281

PMLBCL classification by, 308

PTCL classification by, 373t

PTCL-U classification by, 371

PTLD classification by, 410

SPTCL classification by, 367

THRBCL classification by, 302–303

thymoma classification by, 421, 422t

T-LGL classification by, 330

WM (Waldenstrom macroglobulinemia).
See LPL

Working Formulation, for non-Hodgkin
lymphoma, 42, 45t, 371

World Health Organization. See WHO

Z

Zidovudine (AZT), 10t